SHOUT
SHARE
IT

126901
Sm Inst
12

THE
BIOLOGICAL BULLETIN

PUBLISHED BY
THE MARINE BIOLOGICAL LABORATORY

Editorial Board

GARY N. CALKINS, Columbia University
E. G. CONKLIN, Princeton University
E. N. HARVEY, Princeton University
SELIG HECHT, Columbia University
LEIGH HOADLEY, Harvard University
M. H. JACOBS, University of Pennsylvania
H. S. JENNINGS, Johns Hopkins University
E. E. JUST, Howard University
FRANK R. LILLIE, University of Chicago
CARL R. MOORE, University of Chicago
GEORGE T. MOORE, Missouri Botanical Garden
T. H. MORGAN, California Institute of Technology
G. H. PARKER, Harvard University
W. M. WHEELER, Harvard University
EDMUND B. WILSON, Columbia University

ALFRED C. REDFIELD, Harvard University
Managing Editor

VOLUME LXIX
AUGUST TO DECEMBER, 1935

Smithsonian Institution
304854
National Museum

Printed and Issued by
LANCASTER PRESS, Inc.
PRINCE & LEMON STS.
LANCASTER, PA.

THE BIOLOGICAL BULLETIN is issued six times a year. Single numbers, $1.75. Subscription per volume (3 numbers), $4.50.

Subscriptions and other matter should be addressed to the Biological Bulletin, Prince and Lemon Streets, Lancaster, Pa. Agent for Great Britain: Wheldon & Wesley, Limited, 2, 3 and 4 Arthur Street, New Oxford Street, London, W.C. 2.

Communications relative to manuscripts should be sent to the Managing Editor, Marine Biological Laboratory, Woods Hole, Mass., between June 1 and October 1 and to the Institute of Biology, Divinity Avenue, Cambridge, Mass., during the remainder of the year.

Entered October 10, 1902, at Lancaster, Pa., as second-class matter under
Act of Congress of July 16, 1894.

LANCASTER PRESS, INC., LANCASTER, PA.

CONTENTS

No. 1. August, 1935

PAGE

Thirty-seventh Report of the Marine Biological Laboratory 1

Cleveland, L. R.
The Centrioles of Pseudotrichonympha and their Rôle in Mitosis 46

Cleveland, L. R.
The Intranuclear Achromatic Figure of Oxymonas grandis sp. nov. 54

Yeager, J. Franklin and Oscar E. Tauber
On the Hemolymph Cell Counts of Some Marine Invertebrates 66

Friedmann, Herbert
Notes on Differential Threshold of Reaction to Vitamin D Deficiency in the House Sparrow and the Chick 71

Juday, C., and H. A. Schomer
The Utilization of Solar Radiation by Algae at Different Depths in Lakes 75

Kille, Frank R.
Regeneration in Thyone briareus Lesueur following induced Autotomy 82

Bachem, A., and M. A. Dushkin
A Study of Bacterial Sensitivity to Ultraviolet Radiation 109

Doyle, W. L., and C. W. Metz
Structure of the Chromosomes in the Salivary Gland Cells in Sciara (Diptera) 126

Stump, A. B.
Observations on the Feeding of Difflugia, Pontigulasia and Lesquereusia 136

Turner, Clarence D.
The Effects of Antuitrin-S on the Male Genital Organs of the Lizard (Eumeces laticeps) during Seasonal Atrophy 143

Chase, H. Y.
The Origin and Nature of the Fertilization Membrane in Various Marine Ova 159

PAGE
KAGAN, BENJAMIN M.
The Fertilizable Period of the Eggs of Fundulus heteroclitus and Some Associated Phenomena 185

No. 2. OCTOBER, 1935

ROEHDER, K. D.
An Experimental Analysis of the Sexual Behavior of the Praying Mantis (Mantis religiosa L.) 203
BRAND, THEODOR V.
Methods for the Determination of Nitrogen and Carbon in Small Amounts of Plankton 221
SAYLES, LEONARD P.
The Effects of Salinity Changes on Body Weight and Survival of Nereis virens 233
GRAFFLIN, ALLAN L.
Chloride and Total Osmotic Pressure in the Blood of Marine Teleosts 245
BARNES, T. CUNLIFFE
Salt Requirements and Orientation of Ligia in Bermuda, III. 259
TURNER, C. L.
Studies on the Secondary Sexual Characters of Crayfishes: X. The annulus ventralis in true intersexes of Cambarus 269
HEILBRUNN, L. V., AND R. A. YOUNG
Indirect Effects of Radiation on Sea Urchin Eggs 274
HEILBRUNN, L. V., AND KATHRYN DAUGHERTY
Indirect Effects of Radiation on Elodea Leaves 279
HARVEY, ETHEL BROWNE
The Mitotic Figure and Cleavage Plane in the Egg of Parechinus microtuberculatus, as Influenced by Centrifugal Force 287
HARVEY, ETHEL BROWNE
Some Surface Phenomena in the Fertilized Sea Urchin Egg Influenced by Centrifugal Force 298
HAIG, CHARLES
The Phototropic Responses of Avena in Relation to Intensity and Wave-Length 305
PROGRAM AND ABSTRACTS OF SCIENTIFIC PAPERS
Presented at the Marine Biological Laboratory 325

No. 3. DECEMBER, 1935

RUNNSTRÖM, JOHN
Acid Formation in Frozen and Thawed Arbacia punctulata Eggs and its Possible Bearing on the Problem of Activation .. 345

PAGE

RUNNSTRÖM, JOHN
Influence of Iodoacetate on Activation and Development of the Eggs of Arbacia punctulata 351
GUTTMAN, RITA
Differential Oxygen Uptake of Regions of Limulus Optic Nerve as Related to Distance from the Sense Organ 356
WELLS, NELSON A.
Change in Rate of Respiratory Metabolism in a Teleost Fish Induced by Acclimatization to High and Low Temperature . . 361
SUMNER, F. B., AND N. A. WELLS
Some Relations between Respiratory Metabolism in Fishes and Susceptibility to Certain Anesthetics and Lethal Agents 368
KLEINHOLZ, L. H.
The Melanophore-dispersing Principle in the Hypophysis of Fundulus heteroclitus 379
GRAFFLIN, ALLAN L.
Renal Function in Marine Teleosts, I. 391
CLEMENT, ANTHONY C.
The Formation of Giant Polar Bodies in Centrifuged Eggs of Ilyanassa 403
CHASE, H. Y.
The Effect of Temperature on the Rate of the Fertilization Reaction in Various Marine Ova 415
JOHNSON, MARTIN W.
Seasonal Migrations of the Wood-borer Limnoria lignorum (Rathke) at Friday Harbor, Washington 427
NUTTYCOMBE, JOHN W., AND AUBREY J. WATERS
Feeding Habits and Pharyngeal Structure in Stenostomum . . . 439
DAWSON, J. A., WALTER R. KESSLER, AND JOSEPH K. SILBERSTEIN
Mitosis in Amoeba dubia 447
HUGHES-SCHRADER, SALLY
The Chromosome Cycle of Phenacoccus (Coccidae) 462

Volume LXIX Number 1

THE
BIOLOGICAL BULLETIN

PUBLISHED BY
THE MARINE BIOLOGICAL LABORATORY

Editorial Board

GARY N. CALKINS, Columbia University

E. G. CONKLIN, Princeton University
E. N. HARVEY, Princeton University
SELIG HECHT, Columbia University
LEIGH HOADLEY, Harvard University
M. H. JACOBS, University of Pennsylvania
H. S. JENNINGS, Johns Hopkins University
E. E. JUST, Howard University
FRANK R. LILLIE, University of Chicago
CARL R. MOORE, University of Chicago
GEORGE T. MOORE, Missouri Botanical Garden
T. H. MORGAN, California Institute of Technology
G. H. PARKER, Harvard University
W. M. WHEELER, Harvard University
EDMUND B. WILSON, Columbia University

ALFRED C. REDFIELD, Harvard University
Managing Editor

SMITHSONIAN INSTITUTION
AUG 20 1935
NATIONAL MUSEUM

AUGUST, 1935

Printed and Issued by
LANCASTER PRESS, Inc.
PRINCE & LEMON STS.
LANCASTER, PA.

THE BIOLOGICAL BULLETIN

THE BIOLOGICAL BULLETIN is issued six times a year. Single numbers, $1.75. Subscription per volume (3 numbers), $4.50.

Subscriptions and other matter should be addressed to the Biological Bulletin, Prince and Lemon Streets, Lancaster, Pa. Agent for Great Britain: Wheldon & Wesley, Limited, 2, 3 and 4 Arthur Street, New Oxford Street, London, W.C. 2.

Communications relative to manuscripts should be sent to the Managing Editor, Marine Biological Laboratory, Woods Hole, Mass., between June 1 and October 1 and to the Institute of Biology, Divinity Avenue, Cambridge, Mass., during the remainder of the year.

INSTRUCTIONS TO AUTHORS

Preparation of Manuscript. In addition to the text matter, manuscripts should include a running page head of not more than thirty-five letters. Footnotes, tables, and legends for figures should be typed on separate sheets.

Preparation of Figures. The dimensions of the printed page (4⅜ x 7 inches) should be borne in mind in preparing figures for publication. Drawings and photographs, as well as any lettering upon them, should be large enough to remain clear and legible upon reduction to page size. Illustrations should be planned for sufficient reduction to permit legends to be set below them. In so far as possible, explanatory matter should be included in the legends, not lettered on the figures. Statements of magnification should take into account the amount of reduction necessary. Figures will be reproduced as line cuts or halftones. Figures intended for reproduction as line cuts should be drawn in India ink on white paper or blue-lined coördinate paper. Blue ink will not show in reproduction, so that all guide lines, letters, etc. must be in India ink. Figures intended for reproduction as halftone plates should be grouped with as little waste space as possible. Drawings and lettering for plates should be made directly on heavy Bristol board, not pasted on, as the outlines of pasted letters or drawings appear in the reproduction unless removed by an expensive process. Methods of reproduction not regularly employed by the Biological Bulletin will be used only at the author's expense. The originals of illustrations will not be returned except by special request.

Directions for Mailing. Manuscripts and illustrations should be packed flat between stiff cardboards. Large charts and graphs may be rolled and sent in a mailing tube.

Reprints. Authors will be furnished, free of charge, one hundred reprints without covers. Additional copies may be obtained at cost.

Proof. Page proof will be furnished only upon special request. When cross-references are made in the text, the material referred to should be marked clearly on the galley proof in order that the proper page numbers may be supplied.

Entered October 10, 1902, at Lancaster, Pa., as second-class matter under Act of Congress of July 16, 1894.

Vol. LXIX, No. 1 August, 1935

THE BIOLOGICAL BULLETIN

PUBLISHED BY THE MARINE BIOLOGICAL LABORATORY

THE MARINE BIOLOGICAL LABORATORY

THIRTY-SEVENTH REPORT FOR THE YEAR 1934— FORTY-SEVENTH YEAR

I. TRUSTEES AND EXECUTIVE COMMITTEE (AS OF AUGUST 14 1934) 1
LIBRARY COMMITTEE 3
II. ACT OF INCORPORATION 3
III. BY-LAWS OF THE CORPORATION 3
IV. REPORT OF THE TREASURER 5
V. REPORT OF THE LIBRARIAN 9
VI. REPORT OF THE DIRECTOR 10
Statement 10
Addenda:
1. The Staff, 1934 15
2. Investigators and Students, 1934 18
3. Tabular View of Attendance 27
4. Subscribing and Coöperating Institutions, 1934 28
5. Evening Lectures, 1934 28
6. Shorter Scientific Papers, 1934 29
7. Members of the Corporation 35

I. TRUSTEES

EX OFFICIO

FRANK R. LILLIE, *President of the Corporation,* The University of Chicago.
MERKEL H. JACOBS, *Director,* University of Pennsylvania.
LAWRASON RIGGS, JR., *Treasurer,* 120 Broadway, New York City.
CHARLES PACKARD, *Clerk of the Corporation,* Columbia University.

EMERITUS

H. C. BUMPUS, Brown University.
CORNELIA M. CLAPP, Mount Holyoke College.
E. G. CONKLIN, Princeton University.
C. R. CRANE, New York City.
H. H. DONALDSON, Wistar Institute of Anatomy and Biology.
GILMAN A. DREW, Eagle Lake, Florida.
R. A. HARPER, Columbia University.

AUG 17 1935

M. M. Metcalf, Waban, Mass.
W. B. Scott, Princeton University.
E. B. Wilson, Columbia University.

TO SERVE UNTIL 1938

E. R. Clark, University of Pennsylvania.
Otto C. Glaser, Amherst College.
Ross G. Harrison, Yale University.
E. N. Harvey, Princeton University.
H. S. Jennings, Johns Hopkins University.
F. P. Knowlton, Syracuse University.
Franz Schrader, Columbia University.
B. H. Willier, University of Rochester.

TO SERVE UNTIL 1937

W. R. Amberson, University of Tennessee.
H. B. Goodrich, Wesleyan University.
I. F. Lewis, University of Virginia.
R. S. Lillie, The University of Chicago.
T. H. Morgan, California Institute of Technology.
A. C. Redfield, Harvard University.
C. C. Speidel, University of Virginia.
D. H. Tennent, Bryn Mawr College.

TO SERVE UNTIL 1936

H. B. Bigelow, Harvard University.
R. Chambers, Washington Square College, New York University.
W. E. Garrey, Vanderbilt University Medical School.
Caswell Grave, Washington University.
M. J. Greenman, Wistar Institute of Anatomy and Biology.
A. P. Mathews, University of Cincinnati.
G. H. Parker, Harvard University.
C. R. Stockard, Cornell University Medical College.

TO SERVE UNTIL 1935

W. C. Allee, The University of Chicago.
Gary N. Calkins, Columbia University, *Secretary of the Board of Trustees*
W. C. Curtis, University of Missouri.
B. M. Duggar, University of Wisconsin.
L. V. Heilbrunn, University of Pennsylvania.
W. J. V. Osterhout, Rockefeller Institute for Medical Research.
William M. Wheeler, Harvard University.
Lorande L. Woodruff, Yale University.

Executive Committee of the Board of Trustees

Frank R. Lillie, *Ex Off. Chairman.*
Merkel H. Jacobs, *Ex. Off.*
Lawrason Riggs, Jr., *Ex. Off.*
E. Newton Harvey, to serve until 1935.
C. R. Stockard, to serve until 1935.

G. N. CALKINS, to serve until 1936.
L. V. HEILBRUNN, to serve until 1936.

THE LIBRARY COMMITTEE

E. G. CONKLIN, *Chairman.*
WILLIAM R. AMBERSON.
C. O. ISELIN, II.
C. C. SPEIDEL.
A. H. STURTEVANT.
WILLIAM R. TAYLOR.

II. ACT OF INCORPORATION

No. 3170

COMMONWEALTH OF MASSACHUSETTS

Be It Known, That whereas Alpheus Hyatt, William Sanford Stevens, William T. Sedgwick, Edward G. Gardiner, Susan Minns, Charles Sedgwick Minot, Samuel Wells, William G. Farlow, Anna D. Phillips and B. H. Van Vleck have associated themselves with the intention of forming a Corporation under the name of the Marine Biological Laboratory, for the purpose of establishing and maintaining a laboratory or station for scientific study and investigation, and a school for instruction in biology and natural history, and have complied with the provisions of the statutes of this Commonwealth in such case made and provided, as appears from the certificate of the President, Treasurer, and Trustees of said Corporation, duly approved by the Commissioner of Corporations, and recorded in this office;

Now, therefore, I, HENRY B. PIERCE, Secretary of the Commonwealth of Massachusetts, *do hereby certify* that said A. Hyatt, W. S. Stevens, W. T. Sedgwick, E. G. Gardiner, S. Minns, C. S. Minot, S. Wells, W. G. Farlow, A. D. Phillips, and B. H. Van Vleck, their associates and successors, are legally organized and established as, and are hereby made, an existing Corporation, under the name of the MARINE BIOLOGICAL LABORATORY, with the powers, rights, and privileges, and subject to the limitations, duties, and restrictions, which by law appertain thereto.

Witness my official signature hereunto subscribed, and the seal of the Commonwealth of Massachusetts hereunto affixed, this twentieth day of March, in the year of our Lord One Thousand Eight Hundred and Eighty-Eight.

[SEAL]

HENRY B. PIERCE,
Secretary of the Commonwealth.

III. BY-LAWS OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY

I. The annual meeting of the members shall be held on the second Tuesday in August, at the Laboratory, in Woods Hole, Mass., at 11.30 A.M., daylight saving time, in each year, and at such meeting the members shall choose by ballot a Treasurer and a Clerk to serve one year, and eight Trustees to serve four years. There shall be thirty-two Trustees thus chosen divided into four classes, each to serve four years, and in addition there shall be two groups of Trustees as follows: (*a*) Trustees *ex officio*, who shall be the President of the Corporation, the Director of the Laboratory, the Associate Director, the Treasurer and the Clerk; (*b*) Trustees Emeritus, who shall be elected from the Trustees by the Corporation. Any regular Trustee who has attained the age of seventy years shall continue to serve as Trustee until the next annual meeting of the Corporation, whereupon his office as regular Trustee shall become vacant and be filled by election by the Corporation and he shall become eligible for election as Trustee Emeritus for life. The Trustees *ex officio* and Emeritus shall have all rights of the Trustees except that Trustees Emeritus shall not have the right to vote.

The Trustees and officers shall hold their respective offices until their successors are chosen and have qualified in their stead.

II. Special meetings of the members may be called by the Trustees to be held in Boston or in Woods Hole at such time and place as may be designated.

III. Inasmuch as the time and place of the Annual Meeting of Members is fixed by these By-laws, no notice of the Annual Meeting need be given. Notice of any special meeting of members, however, shall be given by the Clerk by mailing notice of the time and place and purpose of said meeting, at least fifteen (15) days before such meeting, to each member at his or her address as shown on the records of the Corporation.

IV. Twenty-five members shall constitute a quorum at any meeting.

V. The Trustees shall have the control and management of the affairs of the Corporation; they shall present a report of its condition at every annual meeting; they shall elect one of their number President of the Corporation who shall also be Chairman of the Board of Trustees; they shall appoint a Director of the Laboratory; and they may choose such other officers and agents as they may think best; they may fix the compensation and define the duties of all the officers and agents; and may remove them, or any of them, except those chosen by the members, at any time; they may fill vacancies occurring in any manner in their own number or in any of the offices. They shall from time to time elect members to the Corporation upon such terms and conditions as they may think best.

VI. Meetings of the Trustees shall be called by the President, or by any two Trustees, and the Secretary shall give notice thereof by written or printed notice sent to each Trustee by mail, postpaid. Seven Trustees shall constitute a quorum for the transaction of business. The Board of Trustees shall have power to choose an Executive Committee from their own number, and to delegate to such Committee such of their own powers as they may deem expedient.

VII. The accounts of the Treasurer shall be audited annually by a certified public accountant.

VIII. The consent of every Trustee shall be necessary to dissolution of the Marine Biological Laboratory. In case of dissolution, the property shall be disposed of in such manner and upon such terms as shall be determined by the affirmative vote of two-thirds of the Board of Trustees.

IX. These By-laws may be altered at any meeting of the Trustees, provided that the notice of such meeting shall state that an alteration of the By-laws will be acted upon.

X. Any member in good standing may vote at any meeting, either in person or by proxy duly executed.

IV. THE REPORT OF THE TREASURER

To the Trustees of the Marine Biological Laboratory:

Gentlemen: Herewith is my report as Treasurer of the Marine Biological Laboratory for the year 1934.

The accounts have been audited by Messrs. Seaman, Stetson, and Tuttle, certified public accountants, and a copy of their report is on file at the Laboratory and is open to inspection by members of the Corporation.

At the end of the year 1934, the book value of the Endowments Funds in the hands of the Central Hanover Bank and Trust Company as Trustee, was

General Fund,	Securities	$ 908,979.66
	in cash	2,635.71
Library Fund,	Securities	192,820.25
	in cash	994.44
Total book value		$1,105,430.06

The income collected from these Funds was as follows:

General Endowment	$39,394.02
Library Fund	7,543.95

Income in arrears, some of which may never be collected, was on December 31, 1934:

General Fund	$12,966.50
Library Fund	2,897.05

The shrinkage in income from Endowment Funds was more than made up by the extra dividends of the General Biological Supply House, Inc. The income from this stock amounted during the year to $11,834.00.

The *Reserve Fund* was liquidated and the proceeds applied to the further reduction of Devil's Lane mortgage.

Retirement Fund. A net total of $4,060 was paid out of the Retirement Fund in pensions. The fund at the end of the year consisted of

securities of the book value of	$21,847.03
Cash	713.24
Total	$22,560.27

Income in arrears on December 31, was $131.63

The land, building, equipment and library, exclusive of the Gansett and Devil's Lane tracts, represented an investment of	$1,717,498.34
Less reserve for depreciation	412,658.40
	$1,304,839.94

Expenses including $43,427.07 depreciation exceeded income by $15,667.92. There was expended from current funds $16,642.08 in additions to plant, mostly for books.

At the end of the year the Laboratory owed $2,000 on Bond and Mortgage (paid since the end of the year) and $4,760.59 on open account and had $9,937.82 in cash and bank accounts.

Following is the balance sheet as of December 31, 1934, the condensed statement of income and outgo, also the surplus account, as set out by the accountants:

EXHIBIT A

MARINE BIOLOGICAL LABORATORY BALANCE SHEET, DECEMBER 31, 1934

Assets

Endowment Assets and Equities:			
Securities and Cash in Hands of Central Hanover Bank and Trust Company, New York, Trustee—Schedules I-a and I-b		$1,105,430.06	
Securities and Cash—Minor Funds—Schedule II		7,959.34	$1,113,389.40
Plant Assets:			
Land—Schedule IV	$ 98,103.05		
Buildings—Schedule IV	1,224,732.28		
Equipment—Schedule IV	173,840.34		
Library—Schedule IV	220,822.67	$1,717,498.34	
Less Reserve for Depreciation		412,658.40	
		$1,304,839.94	
Cash in Dormitory Building Fund		523.85	
Cash in Reserve Fund		24.65	$1,305,388.44
Current Assets:			
Cash		$ 9,937.82	
Accounts and Notes-Receivable		10,055.61	
Inventories:			
Supply Department	$ 37,589.22		
Biological Bulletin	10,573.93	48,163.15	
Investments:			
Devil's Lane Property	$ 42,991.05		
Gansett Property	5,351.29		
Stock in General Biological Supply House, Inc.	12,700.00		
Retirement Fund Assets	22,560.27	83,602.61	

Prepaid Insurance	3,662.78	
Items in Suspense (Net)	340.24	$ 155,762.21

Liabilities

Endowment Funds:		
Endowment Funds—Schedule III	$1,105,430.06	
Minor Funds—Schedule III	7,959.34	$1,113,389.40
Plant Funds:		
Donations and Gifts—Schedule III	$1,029,572.61	
Other Investments in Plant from Gifts and Current Funds	275,815.83	$1,305,388.44
Current Liabilities and Surplus:		
Accounts-Payable	$ 4,760.59	
Woods Hole Oceanographic Institution	490.94	
Mortgage Note—Devil's Lane Property	2,000.00	
	$ 7,251.53	
Current Surplus—Exhibit C	148,510.68	$ 155,762.21

EXHIBIT B

MARINE BIOLOGICAL LABORATORY INCOME AND EXPENSE, YEAR ENDED DECEMBER 31, 1934

	Total		Net	
	Expense	Income	Expense	Income
Income:				
General Endowment Fund		$ 39,394.02		$ 39,394.02
Library Fund		7,543.95		7,543.95
Instruction	8,030.83	9,825.00		1,794.17
Research	4,012.52	12,237.00		8,224.48
Evening Lectures	55.41		55.41	
Biological Bulletin and Membership Dues	7,786.86	9,113.30		1,326.44
Supply Department—Schedule V	43,428.23	45,439.72		2,011.49
Mess—Schedule VI	21,138.68	21,468.16		329.48
Dormitories—Schedule VII	30,421.08	11,839.13	18,581.95	
(Interest and Depreciation charged to above three Departments—See Schedules V, VI, and VII)	*36,296.75*			36,296.75
Dividends, General Biological Supply House, Inc.		11,834.00		11,834.00
Rents:				
Danchakoff Cottages	377.07	750.00		372.93
Microscopes		530.90		530.90
Newman Cottage	114.40	250.00		135.60
Janitor's House	242.36	360.00		117.64
Sale of Duplicate Library Sets		20.54		20.54
Sundries		9.44		9.44

Maintenance of Plant:				
New Laboratory Expenses	16,008.45		16,008.45	
Chemical and Special Apparatus	12,999.05		12,999.05	
Maintenance, Buildings and Grounds	7,816.95		7,816.95	
Library Department Expenses	7,559.80		7,559.80	
Carpenter Department Expenses	533.83		533.83	
Truck Expenses	731.74		731.74	
Sundry Expenses	309.99		309.99	
Workmen's Compensation Insurance	579.93		579.93	
Pumping Station Expenses	150.76		150.76	
General Expenses:				
Administration Expenses	15,095.21		15,095.21	
Endowment Fund Trustee	968.50		968.50	
Interest on Loans	25.00		25.00	
Bad Debts	452.51		452.51	
Reserve for Depreciation	43,427.07		43,427.07	
Museum Expenses	313.60		313.60	
	$186,283.08	$170,615.16	$125,609.75	$109,941.83
Excess of Expenses over Income carried to Current Surplus—Exhibit C		15,667.92		15,667.92
		$186,283.08		$125,609.75

EXHIBIT C

MARINE BIOLOGICAL LABORATORY, CURRENT SURPLUS ACCOUNT, YEAR ENDED DECEMBER 31, 1934

Balance, January 1, 1934		$130,138.26
Add:		
Reserve for Depreciation charged to Plant Funds		43,427.07
Cash transferred from Reserve Fund and used to reduce Mortgage on Devil's Lane Land		12,000.00
Federal Tax on Checks drawn on Building Fund Cash and charged to Administration Expense		.04
		$185,565.37
Deduct:		
Payments from Current Funds during Year for Plant Assets as shown in Schedule IV:		
Buildings	$ 256.09	
Equipment	3,660.84	
Library, Books, etc.	12,725.15	
	$16,642.08	
Less Cash received for Plant Assets sold and charged to Current Cash	70.00	
	$16,572.08	

Pensions and Allowances Paid	$4,060.00		
Less Income of Retirement Fund received	245.31	3,814.69	
Payment of Balance due on Danchakoff Mortgage from Current Funds		1,000.00	
Excess of Expenses over Income for Year as shown in Exhibit B		15,667.92	37,054.69
Balance, December 31, 1934—Exhibit A			$148,510.68

Respectfully submitted,
LAWRASON RIGGS, JR.,
Treasurer.

V. THE REPORT OF THE LIBRARIAN

The Library budget for 1934 remained the same as that for 1933 and was apportioned as follows: current books, $300; serials, $6,000; current serial binding, $1,500; express, $300; supplies, $500; back sets, $1,850; salaries, $7,150; total, $17,600. The amount spent under each item is here set out: current books, $288.31; serials, $7,388.47; binding, $1,192.24; express, $88.05; supplies, $278.01; back sets, $1,093.86; salaries, $7,150; total, $17,478.94. The total expenditure made for the Woods Hole Oceanographic Institution for their year March 1 to March 1, 1934–35, was approximately as follows: books, $130.60; serials, $229.64; binding, $69.70; supplies, $2.12; back sets, $442.89; total, $874.95.

The Library now contains 38,558 volumes and 86,236 separates. Of the bound volumes 6,036 are books and 32,522 are serials. Of the separates 2,546 are bound or are sizeable volumes and collected reprints. There are received at the Library 1,197 current serials. Of these 349 are subscriptions paid by the Marine Biological Laboratory, 37 by the Woods Hole Oceanographic Institution; 582 are exchanges with the Biological Bulletin, 19 with the Woods Hole Oceanographic Institution publications; and 200 are gifts to the Marine Biological Laboratory, 10 to the Woods Hole Oceanographic Institution. The new books number 170, of which 71 were purchased by the Marine Biological Laboratory, 30 by the Woods Hole Oceanographic Institution; 21 were gifts from publishers, 24 from authors, and 24 were miscellaneous gifts. The new current serials number 32, of which 5 are subscriptions paid by the Marine Biological Laboratory and 8 by the Woods Hole Oceanographic Institution; 13 are exchanges with the Biological Bulletin and 6 with the Woods Hole Oceanographic Institution publications. The new separates received this year number 5,028 and approximately

3,500 are of current issue while the remainder date back three years or more. These older reprints, many of them very old and hard to fill in, were gifts generously made by the following members of the Corporation: Dr. M. E. Collett, Dr. E. O. Jordan, Dr. L. Michaelis, Dr. S. Morgulis, Dr. E. F. Phillips, and Dr. E. B. Wilson.

VI. THE REPORT OF THE DIRECTOR

To the Trustees of the Marine Biological Laboratory:

Gentlemen: I beg to submit herewith a report of the forty-seventh session of the Marine Biological Laboratory for the year 1934.

1. *Attendance.* The attendance both of investigators and students for 1934 shows a slight increase over that for 1933, amounting to 4 for investigators and 13 for students, the total attendance for the two years in question being 439 and 425, respectively. The number of institutions represented by investigators also increased from 120 to 131 and that by students from 58 to 75. The seasonal distribution of the attendance of investigators for 1934, and for comparison that for the preceding 7 years, is indicated in the following table:

		1927	1928	1929	1930	1931	1932	1933	1934
May	30	7	15	9	6	6	8	11	12
June	10	50	64	55	50	51	54	46	54
“	20	114	140	139	153	153	127	129	137
“	30	212	240	197	208	217	172	184	196
July	10	247	281	238	253	258	225	235	249
“	20	247	282	242	250	273	245	253	256
“	30	245	272	249	253	281	248	255	248
August	10	234	250	256	254	302	257	261	264
“	20	208	226	243	245	280	236	244	250
“	30	168	183	220	204	239	190	205	211
September	10	110	112	157	122	136	129	117	93
“	20	50	43	59	44	69	58	45	38
“	30	12	14	14	8	14	13	12	9

2. *The Report of the Treasurer.* The decline in the income from endowment funds of the Laboratory, to which attention was called in the reports for 1932 and 1933, has continued during 1934, though fortunately at a somewhat reduced rate. The amounts received from this source for the four years 1931 to 1935 inclusive (no decline having occurred prior to 1931) were: $57,728.26, $55,668.92, $49,067.17 and $46,937.97. It will be noted that the loss of income from endowment, as based on the 1931 figure, has now reached approximately 19 per cent, which under existing conditions may be considered to indicate a very satisfactory soundness of the investments of the Laboratory as a whole.

The income from the other usual sources remained approximately the same in 1934 as in 1933, further declines in receipts for research space and from the Mess and the dormitories being approximately balanced by gains in those from the Supply Department and from the Courses of Instruction. A dividend from the General Biological Supply House, representing profits accumulated during a number of years, is chiefly responsible for the considerable increase in the total income for 1934 over that for the preceding year. Advantage was taken of this favorable opportunity to pay off, shortly after the period formally covered by this report, the remainder of the Devil's Lane mortgage. Since this mortgage was the only one still outstanding, the property owned by the Laboratory is now free from all encumbrances, and annual payments of interest on mortgages which three years ago amounted to $1,350.00 have been completely eliminated.

It is scarcely necessary to emphasize the fact that the relatively favorable financial showing of the Laboratory in 1934 would have been impossible without a continuation of the drastic economies mentioned in several previous reports. Some of these economies, notably the curtailment of the purchase of books and back sets of journals for the Library and of certain repairs and improvements of buildings and equipment are to be looked upon as emergency measures only, which cannot be long continued without detriment to the scientific activities of the institution.

3. *The Report of the Librarian.* Handicapped both by a reduced appropriation for books and back sets of periodicals and by unfavorable foreign exchange, the Library has nevertheless been able not merely to maintain but slightly to increase the number of journals currently received. At the same time, the valuable reprint collection has continued to grow at an undiminished rate. This growth has been made possible by the many persons who have contributed reprints of their own papers and in particular by those mentioned in the report of the Librarian who have generously made additional gifts. To all these persons the thanks of the Laboratory are hereby extended. The growth of the library during the past 10 years is concisely set forth in the following table:

	1925	1926	1927	1928	1929	1930	1931	1932	1933	1934
Serials received currently .	500	628	764	874	985	1,060	1,080	1,126	1,137	1,197
Total number of bound volumes	15,000	18,200	22,800	26,500	28,300	31,500	33,800	36,000	37,400	38,600
Reprints ...	25,000	38,000	43,000	51,000	59,000	64,000	70,000	76,000	81,000	86,000

4. *Lectures and Scientific Meetings.* A complete record of this important activity of the Laboratory for the season of 1934 will be found below (pages 28 to 35). The regular evening lectures numbered 10, and these were supplemented by several special lectures and exhibits of motion pictures. Ten scientific meetings were also held at which 69 shorter papers were presented and discussed. The last of these meetings was, as usual, an all-day session at the close of the summer, devoted exclusively to work done at the Laboratory during the current season. Abstracts of many of the papers presented at this and at earlier meetings will be found in the Biological Bulletin for October, 1934. In addition to the regular scientific programs, a Woods Hole meeting of the Genetics Society of America on August 22, for which the Marine Biological Laboratory acted as host, was also largely attended by Laboratory workers.

5. *Courses of Instruction.* The resignation of Dr. William R. Amberson as Head of the Physiology Course, a position which he first assumed in 1930, was accepted by the Executive Committee with regret, and Dr. Laurence Irving of the University of Toronto was selected to take his place. In recognition of Dr. Amberson's unusual success during his connection with the Physiology Course in stimulating the interest of students in research, he was elected a member of the Research Staff of the Laboratory.

6. *Change in the By-laws.* At the regular meeting of the Trustees held on Tuesday, August 14, 1934, due notice having previously been given that a change in the By-laws would be acted upon, it was unanimously voted to strike out the former By-law III and to substitute for it a new By-law III as follows:

" Inasmuch as the time and place of the Annual Meeting of Members is fixed by these By-laws, no notice of the Annual Meeting need be given. Notice of any special meeting of members, however, shall be given by the Clerk by mailing notice of the time and place and purpose of said meeting, at least fifteen (15) days before such meeting, to each member at his or her address as shown on the records of the Corporation."

7. *Board of Trustees.* At the meeting of the Corporation held on Tuesday, August 14, 1934, Professor E. G. Conklin, whose long and active service on the Board of Trustees began in 1898, was elected a Trustee Emeritus, and Professor B. H. Willier of the University of Rochester was chosen to fill the vacancy thus created in the Class of 1938.

It is with deep regret that announcement is made of the deaths during 1934 of two members of the Board of Trustees whose connections with the Laboratory were particularly long and intimate.

Dr. Gilman A. Drew, whose death occurred on October 26, first attended the Laboratory as an investigator in 1900. From that year until ill-health necessitated his retirement from active scientific work, he made many important contributions to a better understanding of the marine animals of the Woods Hole region. His services to the Laboratory as an administrator covered almost a quarter of a century, first as Head of the Course in Invertebrate Zoölogy (1901–1909) and later as Assistant Director of the Laboratory (1909–1925). As an elected Trustee and a Trustee ex officio he served from 1907 to 1925 and as a Trustee Emeritus from the latter date until the time of his death. Among the many important services of Dr. Drew to the Marine Biological Laboratory, particular mention should be made of the indispensable part he played in the planning, construction, and equipment of the modern fireproof buildings in which so much of the work of the Laboratory is now carried on. As long as these buildings are used for scientific purposes, investigators will continue to profit by Dr. Drew's foresight, ingenuity, and conscientious attention to details.

Dr. Cornelia M. Clapp was one of the small group of seven persons who attended the Laboratory during its first season in the summer of 1888. Her important scientific work on the toadfish was begun in that year. For a period of 46 years, covering the entire existence of the Laboratory up to the time of her death, her interest in its welfare remained undiminished, and few summers passed without her active participation in its affairs. A member of the Corporation from 1890 until her death on December 31, 1934, she served from 1895 to 1908 as Librarian, from 1897 to 1903 as a member of the Staff of the Course in Embryology, from 1901 to 1904 and from 1910 to 1922 as a Trustee, and since 1922 as a Trustee Emeritus. In her death the Marine Biological Laboratory has sustained an irreparable loss.

8. *Committee of Review.* In the deed of trust covering the endowment funds contributed to the Laboratory in 1924 by the Friendship Fund, Inc., provision was made for a review at 10-year intervals of the activities of the institution by a committee consisting of 9 members in all, including official representatives of the National Academy of Sciences, the National Research Council, the American Association for the Advancement of Science and 6 professors in the field of biology, named respectively by a majority vote of the professors of the departments in the field of biology of the following universities: Harvard University, Columbia University, the University of Chicago, Princeton University, Yale University and the University of Pennsylvania. Provision was further made that if at any time, in the opinion of two-thirds of the members of such a Committee of Review, the Marine Biological

Laboratory is not performing valuable service in biological research, and if, after appropriate notification of the trustees of the fund and the Beneficiary, a second report by the Committee five years later is similarly unfavorable, then the fund may be used for other purposes recommended by two-thirds of the members of the Committee.

The first Committee of Review met at Woods Hole on July 21, 1934. Its members, chosen in the manner prescribed, were Professors G. N. Calkins (Columbia University), Wesley R. Coe (Yale University), Edwin G. Conklin (Princeton University), Frank R. Lillie (The University of Chicago), T. H. Morgan (The American Association for Advancement of Science), Fernandus Payne (The National Research Council), Alfred C. Redfield (Harvard University), Charles R. Stockard (The National Academy of Sciences) and Rodney H. True (The University of Pennsylvania). The Committee, after a study of the work of the Laboratory for the preceding 10 years, based on reports from its President and Director and on exhibits illustrating its attendance and scientific activities during the period in question, reached the following conclusions which were incorporated in the Minutes of the Meeting:

" 1. The organization of the Laboratory, as embodied in its Constitution and By-Laws, continues to operate effectively under the control of professional biologists.

" 2. The large number of investigators who have worked at the Laboratory as shown by Exhibits III and V, drawn as they have been from more than four hundred (400) institutions in this country and in many foreign lands, indicates the breadth of the Laboratory's influence and services.

" 3. The Laboratory is especially well equipped for researches in general biology, experimental zoölogy and botany, embryology, physiology, biochemistry, biophysics, and other branches of the biological sciences. Its research rooms, apparatus, and facilities are excellent, and during the summer these are used to capacity. Living material for research is abundant and is promptly supplied by the Collecting (Supply) Department.

" 4. The Library is generally recognized as one of the best biological libraries in the country; and, as shown by Exhibit VII, has increased about three-fold during the past decennium. It is freely accessible to investigators and is extensively used.

" 5. General lectures and conferences are given at least twice a week throughout the summer session. They are largely attended and in general are very instructive and stimulating.

" 6. One of the most important features of the Laboratory is the close personal association of workers in many fields over considerable periods of time. This is one of the chief attractions of the Marine Biological Laboratory."

The Committee in conclusion voted unanimously that the Marine Biological Laboratory is performing valuable services in biological research.

There are appended as parts of the report:

1. The Staff, 1934.
2. Investigators and Students, 1934.
3. A Tabular View of Attendance, 1930–1934.
4. Subscribing and Coöperating Institutions, 1934.
5. Evening Lectures, 1934.
6. Shorter Scientific Papers, 1934.
7. Members of the Corporation, August, 1934.

Respectfully submitted,
M. H. JACOBS,
Director.

---

1. THE STAFF, 1934

Merkel H. Jacobs, *Director,* Professor of General Physiology, University of Pennsylvania.
Associate Director: ——

ZOÖLOGY

I. Investigation

Gary N. Calkins, Professor of Protozoölogy, Columbia University.
E. G. Conklin, Professor of Zoölogy, Princeton University.
Caswell Grave, Professor of Zoölogy, Washington University.
H. S. Jennings, Professor of Zoölogy, Johns Hopkins University.
Frank R. Lillie, Professor of Embryology, The University of Chicago.
C. E. McClung, Professor of Zoölogy, University of Pennsylvania.
S. O. Mast, Professor of Zoölogy, Johns Hopkins University.
T. H. Morgan, Director of the Biological Laboratory, California Institute of Technology.
G. H. Parker, Professor of Zoölogy, Harvard University.
E. B. Wilson, Professor of Zoölogy, Columbia University.
Lorande L. Woodruff, Professor of Protozoölogy, Yale University.

II. Instruction

T. H. Bissonnette, Professor of Biology, Trinity College.
E. C. Cole, Professor of Biology, Williams College.
B. R. Coonfield, Instructor in Biology, Brooklyn College.
C. E. Hadley, Associate Professor of Biology, New Jersey State Teachers College at Montclair.

S. A. Matthews, Associate in Anatomy, School of Medicine, University of Pennsylvania.
O. E. Nelsen, Instructor in Zoölogy, University of Pennsylvania.
L. P. Sayles, Instructor in Biology, College of the City of New York.

Junior Instructors

F. R. Hayes, Associate Professor of Zoölogy, Dalhousie University.
F. H. Woods, Assistant Professor of Zoölogy, University of Missouri.

PROTOZOÖLOGY

I. Investigation

(*See Zoölogy*)

II. Instruction

Gary N. Calkins, Professor of Protozoölogy, Columbia University.
Rachel Bowling, Instructor in Zoölogy, Columbia University.
Robert W. Stabler, Instructor in Zoölogy, University of Pennsylvania.

EMBRYOLOGY

I. Investigation

(*See Zoölogy*)

II. Instruction

L. G. Barth, Instructor of Experimental Zoölogy, Columbia University.
Hubert B. Goodrich, Professor of Biology, Wesleyan University.
Benjamin H. Grave, Professor of Biology, De Pauw University.
Leigh Hoadley, Professor of Zoölogy, Harvard University. (Absent in 1934.)
Charles Packard, Assistant Professor of Zoölogy, Institute of Cancer Research, Columbia University.
Oscar Schotté, Assistant Professor of Biology, Amherst College.

PHYSIOLOGY

I. Investigation

William R. Amberson, Professor of Physiology, University of Tennessee.
Harold C. Bradley, Professor of Physiological Chemistry, University of Wisconsin.
Walter E. Garrey, Professor of Physiology, Vanderbilt University Medical School.
Ralph S. Lillie, Professor of General Physiology, The University of Chicago.
Albert P. Mathews, Professor of Biochemistry, University of Cincinnati.

II. Instruction

Teaching Staff

William R. Amberson, Professor of Physiology, University of Tennessee.
Robert Chambers, Professor of Biology, New York University.
Rudolf Höber, Visiting Professor of Physiology, University of Pennsylvania.

Laurence Irving, Associate Professor of Physiology, University of Toronto.
Balduin Lucké, Associate Professor of Pathology, University of Pennsylvania.
Leonor Michaelis, Member of the Rockefeller Institute, New York City.

Junior Instructors

A. L. Chute, University of Toronto.
F. J. M. Sichel, New York University.

BOTANY

I. Investigation

C. E. Allen, Professor of Botany, University of Wisconsin.
S. C. Brooks, Professor of Zoölogy, University of California.
B. M. Duggar, Professor of Physiological and Economic Botany, University of Wisconsin.
Ivey F. Lewis, Professor of Biology, University of Virginia.
Wm. J. Robbins, Professor of Botany, University of Missouri.

II. Instruction

William Randolph Taylor, Professor of Botany, University of Michigan.
G. W. Prescott, Assistant Professor of Biology, Albion College. (Absent in 1934.)
Francis Drouet, Research Fellow, University of Missouri.
George J. Hollenberg, Professor of Botany, LaVerne College.

GENERAL OFFICE

F. M. MacNaught, Business Manager.
Polly L. Crowell, Assistant.

RESEARCH SERVICE AND GENERAL MAINTENANCE

Samuel E. Pond, Technical Manager.
Oscar W. Richards, Chemical Service.
G. Failla, X-Ray Physicist.
Thomas E. Larkin, Superintendent.
William Hemenway, Carpenter.
Lester F. Boss, Research Technician.
J. D. Graham, Glassblower.
P. H. Liljestrand, Assistant.

LIBRARY

Priscilla B. Montgomery (Mrs. Thomas H. Montgomery, Jr.), Librarian.
Deborah Lawrence, Secretary.
Doris Endrejat, Mary A. Rohan, Assistants.

SUPPLY DEPARTMENT

James McInnis, Manager.
John J. Veeder, Captain.
Ellis M. Lewis, Engineer.
A. M. Hilton, Collector.
A. W. Leathers, Shipping Department.
Milton B. Gray, Collector.
Geoffrey Lehy, Collector.
Walter Kahler, Collector.

MUSEUM

George M. Gray, Curator.

2. INVESTIGATORS AND STUDENTS, 1934

Independent Investigators

Addison, William H. F., Professor of Normal Histology and Embryology, University of Pennsylvania.
Albaum, Harry G., Fellow in Biology, Brooklyn College.
Allee, W. C., Professor of Zoölogy, The University of Chicago.
Amberson, William R., Professor of Physiology, University of Tennessee.
Anderson, Russell L., Professor of Biology, Johnson C. Smith University.
Appel, F. W., Associate Professor of Biology, St. John's College.
Armstrong, Philip B., Assistant Professor of Anatomy, Cornell University Medical College.
Bailey, Gladys Holmes, Instructor, Hunter College.
Bailey, P. L., Jr., Instructor in Physiology, College of the City of New York.
Baker, Horace B., Associate Professor, University of Pennsylvania.
Ball, Eric G., Associate, Johns Hopkins University Medical School.
Baltzer, F., Professor, University of Berne, Berne, Switzerland.
Barth, L. G., Instructor, Columbia University.
Baxter, James S., Rockefeller Foundation Fellow, Carnegie Institution of Washington.
Beams, H. W., Assistant Professor of Zoölogy, State University of Iowa.
Bernstein, Felix, Visiting Professor, Columbia University.
Bigelow, Robert P., Professor of Zoölogy, Emeritus, Massachusetts Institute of Technology.
Birnie, James H., Graduate Student, Brown University.
Bissonnette, T. Hume, Professor of Biology, Trinity College.
Blanchard, Kenneth C., Associate Professor of Biology, Washington Square College, New York University.
Bogert, L. Jean, Writer of Textbooks on Chemistry and Nutrition.
Bowen, Rufus E., Assistant Professor of Biology, Long Island University.
Bowling, Rachel, Columbia University.
Bradley, H. C., Professor of Physical Chemistry, University of Wisconsin.
Brinley, F. J., Assistant Professor, North Dakota State College.
Brown, Dugald E. S., Assistant Professor of Physiology, New York University and Bellevue Medical College.
Budington, Robert A., Professor of Zoölogy, Oberlin College.
Butler, Elmer G., Associate Professor of Biology, Princeton University.
Buyse, Adrian, Instructor in Zoölogy, University of Rochester.
Calkins, Gary N., Professor of Protozoölogy, Columbia University.
Cannan, Robert Keith, Professor, University and Bellevue Hospital Medical College.
Carothers, E. Eleanor, University of Pennsylvania.
Carpenter, Russell L., Associate in Anatomy, College of Physicians and Surgeons, Columbia University.
Cattell, Ware, Associate Editor, The Scientific Monthly.
Chambers, Robert, Research Professor of Biology, Washington Square College, New York University.
Chen, T. T., Instructor, University of Pennsylvania.
Cheney, R. H., Professor of Biology, Long Island University.
Chidester, F. E., University of Michigan.
Chow, Bacon F., Fellowship, Rockefeller Institute.
Clark, Eleanor Linton, Medical School, University of Pennsylvania.
Clark, Eliot R., Professor of Anatomy, University of Pennsylvania.
Clark, Leonard B., Assistant Professor of Biology, Union College.
Clowes, G. H. A., Director of Research, Lilly Research Laboratories.

Coe, W. R., Professor of Biology, Yale University.
Cohen, Rose S., Teaching Fellow, University of Cincinnati.
Cole, Elbert C., Professor of Biology, Williams College.
Collett, Mary E., Associate Professor of Biology, Western Reserve University.
Conklin, Edwin G., Professor of Biology, Emeritus, Princeton University.
Coonfield, B. R., Instructor, Brooklyn College.
Copeland, Manton, Professor of Biology, Bowdoin College.
Costello, Donald P., Instructor in Zoölogy, University of Pennsylvania.
Cowles, Rheinart P., Professor of Zoölogy, Johns Hopkins University.
Crampton, Henry E., Professor of Zoölogy, Barnard College, Columbia University.
Croasdale, Hannah T., Graduate Student, University of Pennsylvania.
Crowell, Prince Sears, Jr., Graduate Student and Assistant in Zoölogy, Harvard University.
Dan, Katsuma, Graduate Student, University of Pennsylvania.
Danforth, Louise L., American University.
Danielli, James F., Princeton University.
Davis, James E., Research Assistant, The University of Chicago.
Diller, William F., Instructor in Zoölogy, Dartmouth College.
Donaldson, Henry H., Member, Wistar Institute.
Drouet, Francis, In Charge of the Herbarium, University of Missouri.
DuBois, Eugene F., Professor of Medicine, Cornell University Medical College.
Duggar, B. M., Professor of Plant Physiology and Applied Botany, University of Wisconsin.
Dunning, Wilhelmina F., Associate in Cancer Research, Institute of Cancer Research, Columbia University.
Dunn, Edwin E., Instructor in Biochemistry, University of Nebraska, College of Medicine.
Duryee, William R., Instructor in Zoölogy, Northwestern University.
Edwards, Dayton J., Associate Professor of Physiology, Cornell University Medical College.
Ephrussi, Boris, Fellow, Rockefeller Foundation.
Ets, Harold N., Associate Professor, Pharmacology, Loyola University School of Medicine.
Everett, John W., Instructor in Anatomy, Duke University.
Fahnestock, Margaret, Instructor, Northwestern University.
Fleisher, Moyer S., Professor of Bacteriology and Hygiene, St. Louis University.
Forbes, Grace S., Instructor in Zoölogy, Barnard College, Columbia University.
Fowler, Coleen, Graduate Student, Johns Hopkins University.
Francis, W. L., Fellow, Rockefeller Foundation.
Fry, Henry J., Visiting Investigator, Cornell University Medical College.
Fuchs, Barrett, American University.
Furth, Jacob, Assistant Professor in Pathology, Cornell University Medical College.
Furtos, Norma C., Fellow in Zoölogy, Western Reserve University.
Garrey, W. E., Professor of Physiology, School of Medicine, Vanderbilt University.
Goddard, David R., National Research Council Fellow, Rockefeller Institute.
Goldforb, A. J., Professor of Biology, College of the City of New York.
Goodrich, H. B., Professor of Biology, Wesleyan University.
Graubard, Marc A., Columbia University.
Grave, B. H., Professor of Zoölogy, DePauw University.
Grave, Caswell, Professor of Zoölogy, Washington University.
Green, David E., Medical Fellow, Cambridge University.
Hadley, Charles E., Associate Professor of Biology, Montclair State Teachers College.

Hall, F. G., Professor of Zoölogy, Duke University.
Harnly, Marie L., Assistant in Biology, Washington Square College, New York University.
Harnly, Morris H., Assistant Professor, Washington Square College, New York University.
Hartline, H. K., Fellow in Medical Physics, University of Pennsylvania.
Harvey, E. Newton, Professor of Physiology, Princeton University.
Harvey, Ethel B., Princeton University.
Hayes, F. Ronald, Associate Professor of Zoölogy, Dalhousie University.
Haywood, Charlotte, Associate Professor of Physiology, Mount Holyoke College.
Heilbrunn, L. V., Associate Professor of Zoölogy, University of Pennsylvania.
Höber, Josephine, University of Pennsylvania.
Höber, Rudolf, Visiting Professor, University of Pennsylvania.
Hooker, Charles W., National Research Council Fellow, University of Rochester.
Howe, H. E., Editor, Industrial and Engineering Chemistry.
Irving, Laurence, Associate Professor of Physiology, University of Toronto.
Jacobs, M. H., Professor of General Physiology, University of Pennsylvania.
Jastrow, Joseph, Emeritus Professor of Psychology, University of Wisconsin.
Jenkins, George B., Professor of Anatomy, George Washington University.
Johlin, J. M., Associate Professor of Biochemistry, Vanderbilt Medical School.
Jollos, Victor, Visiting Professor of Zoölogy and Genetics, University of Wisconsin.
Jones, Edgar P., Instructor, University of Akron.
Jones, Ruth McClung, Instructor in Biology, Swarthmore College.
Kagan, Benjamin M., Johns Hopkins University Medical School.
Kagan, H. E., West Virginia University.
Kaiser, Samuel, Tutor in Biology, Brooklyn College.
Keil, Elsa M., Instructor in Zoölogy, New Jersey College for Women.
Keyes, Donald B., Professor of Chemical Engineering, University of Illinois.
Kidder, George W., Tutor, College of the City of New York.
Kille, Frank R., Instructor in Zoölogy, Swarthmore College.
Kilpatrick, Martin, Assistant Professor of Chemistry, University of Pennsylvania.
Kilpatrick, Mary L., University of Pennsylvania.
Kirkpatrick, T. Bruce, Associate Professor of Physical Education, Columbia University.
Kleinholz, Lewis H., Graduate Student, Harvard University.
Knower, Henry McE., Research Associate in Biology, Yale University.
Knowlton, Frank P., Professor of Physiology, College of Medicine, Syracuse University.
Lancefield, D. E., Associate Professor of Zoölogy, Columbia University.
Laug, Edwin P., Fellow in Chemistry, University of Tennessee.
Levy, Milton, Assistant Professor of Chemistry, University and Bellevue Hospital Medical College.
Liedke, Kathe R., Graduate Student, Columbia University.
Lillie, Frank R., Chairman of the Department of Zoölogy, The University of Chicago.
Lillie, Ralph S., Professor of General Physiology, The University of Chicago.
Lippay-Wastl, Helene, Head of Department of Physiology, Woman's Medical College of Pennsylvania.
Livingston, Luzern C., National Research Council Fellow in Botany, Bussey Institution, Harvard University.
Loehwing, W. F., Professor of Botany, State University of Iowa.
Lucke, Balduin, Professor of Pathology, University of Pennsylvania, School of Medicine.

MacCardle, Ross C., Instructor in Zoölogy, Temple University.
Magers, Elizabeth, Assistant Professor of Physiology, Vassar College.
Magruder, Samuel R., Assistant, University of Cincinnati.
Margolis, Otto S., Assistant, Washington Square College.
Marsland, Douglas, Assistant Professor of Biology, New York University.
Martin, Earl A., Chairman, Department of Biology, Brooklyn College.
Mathews, A. P., Professor of Biochemistry, University of Cincinnati.
Matthews, Samuel A., Associate in Anatomy, University of Pennsylvania.
Mavor, James W., Professor of Biology, Union College.
Michaelis, Leonor, Member, Rockefeller Institute.
Mirsky, Alfred E., Associate in Medicine, Rockefeller Institute.
Moment, Gairdner B., Instructor, Goucher College.
Morgan, Lilian V., California Institute of Technology.
Morgan, T. H., Professor of Biology, California Institute of Technology.
Morrill, Charles V., Associate Professor, Cornell University Medical College.
Nabrit, S. M., Chairman, Department of Biology, Atlanta University.
Nasset, E. S., Instructor in Physiology, University of Rochester.
Nelsen, O. E., Instructor in Zoölogy, University of Pennsylvania.
Nicholas, Warren W., 65 Broadway, New York City, New York.
Nonidez, José F., Assistant Professor of Anatomy, Cornell University Medical College.
Northrop, John H., Member, The Rockefeller Institute.
Novikoff, Alex B., Tutor, Brooklyn College.
Osterhout, W. J. V., Member, Rockefeller Institute for Medical Research.
Orr, Paul R., Instructor in Biology, Brooklyn College.
Packard, Charles, Assistant Professor, Institute of Cancer Research, Columbia University.
Parker, George H., Professor of Zoölogy, Harvard University.
Parpart, Arthur K., Assistant Professor, Princeton University.
Pelluet, Dixie, Assistant Professor of Zoölogy, Dalhousie University.
Plough, Harold H., Professor of Biology, Amherst College.
Pollister, Arthur W., Instructor in Zoölogy, Columbia University.
Pond, Samuel E., Technical Manager, Marine Biological Laboratory.
Rand, Herbert W., Associate Professor of Zoölogy, Harvard University.
de Renyi, George S., Associate Professor of Anatomy, University of Pennsylvania.
Reznikoff, Paul, Assistant Professor of Medicine, Cornell University Medical College.
Richards, Oscar W., Instructor in Biology, Yale University.
Robertson, C. W., Assistant in Biology, Washington Square College, New York University.
Root, Walter S., Associate Professor of Physiology, College of Medicine, Syracuse University.
Rugh, Roberts, Instructor, Hunter College.
Rundles, Wayne, Assistant in Anatomy, Cornell University.
Runnström, John, Professor, University of Stockholm.
Saslow, George, Assistant Professor of Biology, New York University.
Sayles, Leonard P., Instructor, College of the City of New York.
Schechter, Victor, Tutor in Biology, College of the City of New York.
Schmidt, Ida Genther, Research Assistant and Instructor in Histology, Children's Hospital and University of Cincinnati.
Schmidt, Leon H., Research Fellow in Biochemistry, Christ Hospital and University of Cincinnati.
Schotté, Oscar E., Assistant Professor of Zoölogy, Amherst College.
Scott, Allan C., Assistant in Zoölogy, Columbia University.
Shaw, Myrtle, Junior Bacteriologist, New York State Department of Health.

Shumway, Waldo, Professor of Zoölogy, University of Illinois.
Sichel, Ferdinand J. M., Instructor in Zoölogy, University of Pennsylvania.
Slifer, Eleanor H., Associate, State University of Iowa.
Solberg, Archie N., Assistant in Zoölogy, Columbia University.
Sonneborn, Tracy M., Associate, Johns Hopkins University.
Specht, H., Adam T. Bruce Fellow in Zoölogy, Johns Hopkins University.
Speicher, Kathryn Gilmore, Instructor, Pennsylvania College for Women.
Speidel, Carl C., Professor of Anatomy, University of Virginia.
Stabler, Robert M., Instructor in Zoölogy, University of Pennsylvania.
Stancati, Milton F., Graduate Student, University of Pittsburgh.
Steinbach, Henry B., National Research Fellow in Biology, The University of Chicago.
Stewart, Dorothy R., Assistant Professor of Biology, Skidmore College.
Stockard, C. R., Professor of Anatomy, Cornell University Medical College.
Stokey, Alma G., Professor, Mount Holyoke College.
Strong, Oliver S., Professor of Neurology and Neuro-Histology, College of Physicians and Surgeons, Columbia University.
Stuck, Florence, Graduate Student, Columbia University.
Stunkard, H. W., Professor of Biology, New York University.
Sumwalt, Margaret, National Research Fellow, University of Pennsylvania.
Tashiro, Shiro, Professor of Biochemistry, University of Cincinnati.
Taylor, George W., National Research Fellow, Princeton University.
Taylor, William R., Professor of Botany, University of Michigan.
Troisi, Raphael A., Instructor, Temple University.
Tulloch, George S., Instructor, Brooklyn College.
Weisman, Maxwell N., Tutor of Biology, College of the City of New York.
Whedon, Arthur D., Professor of Zoölogy, North Dakota State College.
Whiting, Anna R., Professor of Biology, Pennsylvania College for Women.
Whiting, P. W., Carnegie Institution of Washington, Cold Spring Harbor, N. Y.
Wieman, H. L., Professor of Zoölogy, University of Cincinnati.
Williams, R. G., Assistant Professor of Anatomy, University of Pennsylvania.
Willier, B. H., Professor of Zoölogy, University of Rochester.
Wilson, E. B., Professor Emeritus in Residence, Columbia University.
Witschi, Emil, Professor of Zoölogy, State University of Iowa.
Wodehouse, R. P., Director Hayfever Department, The Arlington Chemical Company.
Wolf, E. Alfred, Associate Professor, University of Pittsburgh.
Woodruff, Lorande L., Professor of Protozoölogy, Yale University.
Woods, Farris H., Assistant Professor of Zoölogy, University of Missouri.
Woodward, Alvalyn E., University of Michigan.
Yeager, J. Franklin, Associate Professor, Iowa State College.
Young, Roger A., Assistant Professor, Howard University.
Zeleny, Charles, Professor of Zoölogy, University of Illinois.
Zirkle, Conway, Associate Professor, University of Pennsylvania.

Beginning Investigators

Abramowitz, A. A., Austin Teaching Fellow in Zoölogy, Harvard University.
Angerer, C. A., Harrison Fellow in Zoölogy, University of Pennsylvania.
Bosworth, Millard W., Research Assistant, Wesleyan University.
Buck, John B., Assistant in Zoölogy, Johns Hopkins University.
Butler, Thomas C., Research Assistant, Vanderbilt University Medical School.
Carlson, J. Gordon, Instructor in Biology, Bryn Mawr College.
Chao, Iping, Graduate Student, The University of Chicago.
Churney, Leon, Graduate Student, University of Pennsylvania.
Clark, Jean M., Graduate Student in Zoölogy, University of Pennsylvania.
Clement, A. C., Graduate Student, Princeton University.

Derrickson, Mary B., Assistant, Vassar College.
Drumtra, Elizabeth, Assistant in Zoölogy, Barnard College.
Engel, Frank L., Dartmouth College.
Engel, George L., Student, Dartmouth College.
Fisher, Saul H., Student, University and Bellevue Hospital Medical College.
Frank, John A., Columbia University.
Gubner, Richard, Student, University and Bellevue Hospital Medical College.
Hollingsworth, Josephine, Graduate Student, University of Pennsylvania.
Hopkins, Florian, Teaching Fellow, University of Tennessee.
Hunter, Francis R., Laboratory Assistant, Princeton University.
Hunter, Laura N., Graduate Student, University of Pennsylvania.
Jones, Roy W., Associate Professor of Biology, Central State Teachers College.
Kaliss, Nathan, Assistant in Zoölogy, Columbia University.
Keller, Thelma C., Graduate Student, Columbia University.
Keltch, Anna K., Research Assistant, Lilly Research Laboratories.
Krahl, Maurice E., Research Assistant, Lilly Research Laboratories.
Lansing, Albert I., Student, University of Pennsylvania.
McLane, Kathryn, Instructor in Biology, Annapolis High School.
Mast, Louise R., Graduate Student, Johns Hopkins University.
Maxwell, Jane, Instructor, Carnegie Institute of Technology.
Mazia, Daniel, University of Pennsylvania.
Miller, James A., Jr., Graduate Assistant, The University of Chicago.
Miller, Roland E., Graduate Assistant, University of Pittsburgh.
Morris, Samuel, Graduate Student, University of Pennsylvania.
Moser, Floyd, Graduate Student, University of Pennsylvania.
Peabody, Elizabeth B., Graduate Student, Radcliffe College.
Redmond, Albert C., Student Assistant, Cornell University Medical College.
Shapiro, Herbert, National Research Fellow, Princeton University.
Stanbury, John, Duke University.
Stirling, Arthur C., Student, Princeton University.
Stone, Faith, Fellow in Department of Zoölogy, The University of Chicago.
Summers, Francis M., Graduate Student, Columbia University.
Tonks, Robert E., University of Pennsylvania.
Townsend, Grace, Graduate Student, The University of Chicago.
Verwey, Willard F., Graduate, Rutgers University.
Webster, Edward C., Teaching Fellow, New York University.
Wichterman, Ralph, Graduate Student, University of Pennsylvania.
Wilbrandt, Walther, Travelling Fellow, Rockefeller Foundation.
Wilson, Hildegard, Assistant in Chemistry, University and Bellevue Hospital Medical College.

Research Assistants

Adams, Philip D., Research Assistant, University of Cincinnati.
Armstrong, Louise S., Cornell University Medical College.
Averill, Charles, Undergraduate, Amherst College.
Beck, Lyle V., Lilly Research Laboratories, Indianapolis, Indiana.
Bengel, Walter Z., Assistant, DePauw University.
Bitterlick, Gerhard, Chemist, Rockefeller Institute.
Bradley, E. Morton, Teaching Assistant, University of Rochester.
Brambel, C. E., Instructor, Johns Hopkins University.
Collings, Doyne, Assistant in Zoölogy, DePauw University.
Corson, Samuel A., Research Assistant, University of Pennsylvania.
Cunningham, Mary Alice, Assistant, DePauw University.
Eagle, Harry, Associate, University of Pennsylvania Medical School.
Evans, Gertrude, Research Assistant, The University of Chicago.
Fraser, Doris A., Research Fellow, University of Pennsylvania.

Fuchs, Herbert A., American University.
Glassman, Harold N., University of Pennsylvania.
Godrich, Jack, Research Assistant, Columbia University.
Grand, C. G., Research Assistant, Washington Square College, New York University.
Hamilton, Mary Alice, Graduate Student, Columbia University.
Hill, Edgar S., Research Assistant, Rockefeller Institute.
Hollenberg, George J., Professor of Biology, LaVerne College.
Howe, Clarissa R., Undergraduate, Goucher College.
Jao, Chin-Chih, University Fellowship, University of Michigan.
Kondritzer, Albert A., Graduate Student, University of Cincinnati.
Landowne, Milton, Student, Harvard University Medical School.
Larrabee, Martin G., Johnson Foundation, University of Pennsylvania.
Lippman, Richard W., Undergraduate, Yale University.
Manery, Jeanne F., Fellow in Physiology, University of Toronto.
Moreland, Ferrin B., Assistant in Biochemistry, Vanderbilt University Medical School.
Naumann, Rudolph V., Assistant, Yale University.
Nestler, Herbert A., Substitute in Biology, Brooklyn College.
Oesting, R. B., Research Assistant, The University of Chicago.
Porter, Helen, Laboratory Assistant, Harvard University.
Rawles, Mary E., Research Assistant, University of Rochester.
Richardson, Margaret S., Research Assistant, Cornell University Medical College.
Robertson, Lola E., Research Assistant, New York University.
Robinson, Miles H., Graduate Student, Swarthmore College.
Runnström, Astri, University of Stockholm.
Schlumberger, Hans G., University of Pennsylvania Medical School.
Schweitzer, Morton D., Assistant in Zoölogy, Columbia University.
Shaw, Isidor, Research Assistant, Long Island University.
Solandt, Donald Y., Research Associate, University of Toronto.
Solandt, Omond McK., Research Associate, University of Toronto.
Speicher, B. R., Carnegie Institution of Washington.
Stricker, George J., Student, Yale University.
Stueck, George H., Jr., Teaching Fellow in Chemistry, New York University.
Tauber, Oscar E., Research Fellow, Iowa State College.
Wade, Lucille W., Research Assistant, Lilly Research Laboratories.
Webster, Margaret D., Dalhousie University.
Wharton, Marguerite, Graduate Assistant, Montclair State Teachers College.
Whitman, B. L., Student, University of Pennsylvania.
Young, Saul B., Rockefeller Institute for Medical Research.

Students

BOTANY

Almstedt, Margaret F., University of Missouri.
Chute, Hettie M., Assistant Professor of Botany, New Jersey College for Women.
Cohen, Aaron A., Student, Harvard University.
Cohn, Julius, 1987 Davidson Avenue, Bronx, New York.
Davidheiser, Cedric B., Swarthmore College.
Hirsch, Dorothy, Laboratory Assistant, New York University.
Johnson, Karl R., High School Instructor, Galesburg, Illinois.
Kershaw, Margaret A., Student, Wheaton College.
Krueger, Lillian K., Teacher, Scott High School.
Plitt, Thora M., Instructor, Vassar College.

Sielke, Dorothy H., Instructor in Botany, Hunter College.
Smith, Arlo I., Graduate Assistant, Northwestern University.
Stewart, Sara R., Wellesley College.

EMBRYOLOGY

Andrew, Warren, Graduate Student, Yale University.
Bartholomew, Olive F., Radcliffe College.
Beeson, Rose M., Assistant, H. Sophie Newcomb College.
Blagg, Amy E., Assistant Professor of Zoölogy, Grinnell College.
Bradley, Encil M., Jr., Graduate Teaching Assistant, University of Rochester.
Collings, William D., Undergraduate Assistant, DePauw University.
Compton, Alfred D., Jr., Princeton University.
Cotton, Cornelia M., Instructor in Zoölogy, American University.
DeLamater, Doan E., Student, Johns Hopkins University.
Dewey, Virginia C., Simmons College.
Doan, Dorothy A., Barnard College.
Flynn, Carl M., Instructor in Zoölogy, University of Maine.
Forster, Margaret, 61 Anderson Street, Boston, Massachusetts.
Foster, Dorothy L., Oberlin College.
Hamilton, Dorothy B., Graduate Research Assistant, Western Reserve University.
Hughes, Roscoe D., Assistant in Zoölogy, Columbia University.
Ives, Philip T., Research Fellow, Amherst College.
Jackson, Floretta A., Assistant in Zoölogy, Barnard College, Columbia University.
Jones, Lawrence M., Student, University of Colorado.
Kammeraad, Adrian, Graduate Student, Yale University.
Knight, Betty L., Technician, Rockefeller Institute.
Mohr, John L., Graduate Student, Oberlin College.
Moore, Caroline, University of Pennsylvania.
Porter, Homer C., Instructor in Biology and Geology, Washington and Jefferson College.
Robinson, Miles H., Swarthmore College.
Sabin, Leonore, Student, Hunter College.
Spofford, Walter R., II, Graduate Student, Yale University.
Sumner, Ruth, Instructor, University of Colorado.
Tiller, Martha G., Teacher Public Schools, Birmingham.
Williams, Margarita, Research Assistant, University of Iowa.

PHYSIOLOGY

Appel, Frederick W., Associate Professor of Biology, St. John's College.
Averill, Charles, Student, Amherst College.
Bosworth, Millard W., Denison Research Assistant, Wesleyan University.
Campbell, Mildred L., Assistant, Mount Holyoke College.
Cortell, Ruth E., Student, Wellesley College.
Dickey, Joanna, Milton Academy.
Ets, Harold N., Professor of Pharmacology, Loyola Medical School.
Goggio, Alfred, Student, University of Toronto.
Hardy, James D., Physicist, Russell Sage Institute of Pathology.
Hechenbleikner, Herbert, Harvard University.
Hollingsworth, Josephine, Graduate Student, University of Pennsylvania.
Holzer, Franz J., Innsbruck, Austria.
Hopkins, Florian G., Teaching Fellow, University of Tennessee.
Hrubetz, M. Caroline, Instructor in Physiology and Pharmacology, College of Physicians and Surgeons.

Hunter, Francis R., Wesleyan University.
Kohn, Henry I., Assistant, Harvard University.
Kuyper, Adrian C., Instructor, State University of Iowa.
McLane, Kathryn, Teacher, Annapolis High School.
Neal, Mary E., Teacher, City of Boston.
Peskin, James C., Rutgers University.
Rose, Sylvan M., Graduate Assistant, Amherst College.
Stanbury, John B., Student, Duke University.
Verwey, Willard F., Rutgers University.

PROTOZOÖLOGY

Averbach, Chemda, Columbia University.
Foster, Edith F., Vassar College.
Frees, Mary K., Student, Columbia University.
Greene, Margery, Student, Columbia University.
Johnson, Arlene C., Graduate Student, Columbia University.
Kahn, Ephraim M., Student, Harvard University.
Liming, Rosamond M., Student, New Jersey College for Women.
Lippman, Richard W., Student, Yale University.
Middleton, Cora D., Student, Columbia Teachers College.
Milne, Lorus J., Student, University of Toronto.
Resnick, Rose, Student, Hunter College.

INVERTEBRATE ZOÖLOGY

Andrews, S. Ralph, Jr., American University.
Barton, Eleanor P., Head of Science Department, Edgewood Park Jr. College.
Beard, Raimon L., Undergraduate Assistant, Wesleyan University.
Bengel, Walter Z., Undergraduate, DePauw University.
Berliner, Robert W., Undergraduate, Yale University.
Blood, Rozella, University of Kansas.
Blouin, Alexander, Professor of Biology, Mount St. Louis College.
Bolt, Otto A., Instructor, Negaunee High School.
Bragg, James H., Student, Central State Teachers College.
Bridges, Martha A., DePauw University.
Bridgman, Jane, Student, Smith College.
Browman, Ludwig G., Curator, The University of Chicago.
Bumpus, Dean F., Student, Oberlin College.
Caldwell, Billie, Union City, Tennessee.
Chen, Yie, Graduate Student, University of Pennsylvania.
Crouse, Helen V., Goucher College.
Danforth, Louise L., Graduate Student, American University.
Dansereau, Antonio, Priest Professor, Montreal College.
Eckels, Richard P., Graduate Student, University of Virginia.
Ettensperger, Flora, Barnard College.
Gay, Elizabeth H., Graduate Student, Johns Hopkins University.
Gilbert, Perry W., Instructor, Dartmouth College.
Glass, Manuel, Yale University.
Gross, Charlotte C., Teacher, Union High School, New Jersey.
Hadley, Ruth G., Leonia, New Jersey.
Hierholzer, Carolyn Anne, Undergraduate, New Jersey College for Women.
Jailer, Joseph W., College of the City of New York.
Jakobsen, Edith M., Biology Assistant, New Jersey State Teachers College.
Kennedy, Kathleen M., St. Francis Xavier University.
Leach, Eleanor, Curator of the Laboratories, Wellesley College.

Leist, Claude, Instructor in Biology, Kansas State Teachers College.
Martin, Mary Louise, Student, Pennsylvania College for Women.
Merrill, Julia E., Student, Wilson College.
Mitchell, Helen U., Student, Hood College.
Mizelle, John D., Assistant, University of Illinois.
Morland, Hazel M., Swarthmore College.
Ormsby, Louise, Columbia University.
Perry, John H., Student, Acadia University.
Pettingill, Olin S., Jr., Instructor, Bowdoin College.
Pomerat, Gerard R., Clark University.
Roney, Harland B., Laboratory Assistant in Zoölogy, University of Illinois.
Rositzke, Marion M., Hunter College.
Saunders, Barbara, Radcliffe College.
Schoenheit, Dorothy, Student, University of Rochester.
Schultz, Helen H., Assistant Professor of Biology, State Teachers College.
Simpson, Alan Gilbert, Jr., Undergraduate Assistant, Wesleyan University.
Smith, Maurice A., Wabash College.
Swartz, Daphne B., Assistant Professor of Biology, Bradley Institute.
Thayer, Mary J., Yale University.
Tyson, Rebecca J., Student, Wayne University.
Weishaar, Irma R., Instructor, Hunter College.
Welch, Dorothy I., Student, Radcliffe College.
Wharton, George W., Jr., Duke University.
Wightman, John C., Student, Oberlin College.

3. TABULAR VIEW OF ATTENDANCE

	1930	1931	1932	1933	1934
INVESTIGATORS—Total	337	362	314	319	323
Independent	217	236	212	210	222
Under Instruction	87	83	73	66	49
Research Assistants	33	43	29	43	52
STUDENTS—Total	136	125	132	118	131
Zoölogy	56	55	55	54	54
Protozoölogy	14	17	16	11	11
Embryology	27	29	29	28	30
Physiology	23	17	18	19	23
Botany	16	7	14	6	13
TOTAL ATTENDANCE	473	487	446	437	454
Less Persons registered as both students and investigators	14	20	14	12	15
	459	467	432	425	439
INSTITUTIONS REPRESENTED—Total	126	137	141	120	131
By Investigators	95	102	94	92	98
By Students	71	68	76	58	75
SCHOOLS AND ACADEMIES REPRESENTED					
By Investigators	–	–	–	1	1
By Students	4	4	1	2	5
FOREIGN INSTITUTIONS REPRESENTED					
By Investigators	7	8	8	5	4
By Students	2	1	1	–	1

4. SUBSCRIBING AND COOPERATING INSTITUTIONS IN 1934

American University
Amherst College
Atlanta University
Barnard College
Berea College
Bowdoin College
Bryn Mawr College
Chinese Educational Mission
College of St. Elizabeth
Columbia University
Commonwealth Fund
Cornell University
Cornell University Medical College
Dalhousie University
DePauw University
Duke University
General Education Board
Goucher College
Harvard University
Harvard University Medical School
Hunter College
Industrial & Engineering Chemistry, of the American Chemical Society
Iowa State College
Johns Hopkins University
Johns Hopkins University Medical School
Johnson C. Smith University
Eli Lilly & Co.
Long Island University
Morehouse College
Mount Holyoke College
New York State Department of Health
New York University
New York University Medical School
Northwestern University
Oberlin College
Pennsylvania College for Women
Princeton University
Radcliffe College
Rockefeller Foundation
Rockefeller Institute for Medical Research
Rutgers University
Smith College
Sophie Newcomb College
Swarthmore College
Syracuse University
Temple University
Tufts College
University of Chicago
University of Cincinnati
University of Illinois
University of Iowa
University of Kansas
University of Michigan
University of Pennsylvania
University of Pennsylvania Medical School
University of Pittsburgh
University of Rochester
University of Rochester Medical School
University of Virginia
Vanderbilt University Medical School
Vassar College
Wabash College
Wellesley College
Wesleyan University
Wheaton College
Wilson College
Wistar Institute of Anatomy and Biology
Yale University

5. EVENING LECTURES, 1934

Friday, June 29
Dr. W. R. Taylor .... "A Biologist's Impressions of the Galapagos Islands."

Friday, July 6
Dr. William R. Amberson ...... "On the Significance of the Vertebrate Erythrocyte."

Friday, July 13
Dr. B. H. Willier .... “An Experimental Study of the Embryonic Differentiation of the Sex-gland and Germ-cells.”

Friday, July 20
Dr. John Runnström .... “Initiation of Development and the Metabolism of the Sea-urchin Egg.”

Friday, July 27
Dr. Rudolf Höber .... “Experiments on the Osmotic Properties of Glands.”

Wednesday, August 8
Dr. Irving Langmuir .... “Monomolecular Films at the Interface between Oil and Water.”

Friday, August 10
Dr. Henry E. Crampton .... “Studies on Evolution in the Islands of the South Seas.”

Friday, August 17
Dr. Lee Foshay .... “Studies on Alteration of Virulence of Bacterium tularense.”

Thursday, August 23
Dr. F. Baltzer .... “Experiments on Sex-development in Bonellia.”

Tuesday, August 28
Dr. C. G. Hartman .... “The Time of Ovulation in the Menstrual Cycle.”

SPECIAL LECTURES AND MOTION PICTURES

Thursday, July 26
Dr. Robert Chambers and
Mr. C. G. Grand .... “Motion Pictures of Microdissection and of Experimental Studies on Segmentation of Sea Urchin Eggs.”

Friday, August 10
Dr. Horace W. Stunkard .... “Life Cycles of Digenetic Trematodes.”

Wednesday, August 15
Dr. E. R. and Mrs. E. L. Clark .. “Motion Pictures on Observations on the Formation of Arteriovenous Anastomoses.”
Dr. C. C. Speidel .... “Motion Pictures on Experimental Study of Striated Muscle in Vivo.”

6. SHORTER SCIENTIFIC PAPERS, 1934

Tuesday, July 3	
Dr. Mary E. Collett	“Ovarian Hormones and Basal Metabolism.”
Dr. Laurence Irving and Mrs. Mary Scott Welch	“The Carbon Dioxide Tension in the Brain.”
Dr. E. S. Nasset	“A Method for the Estimation of Changes in the Rate of Intestinal Secretion.”
Dr. G. H. Parker	“Transfer of Neurohumors.”
Tuesday, July 10	
Dr. Ethel Browne Harvey	“Some Phenomena Produced by Centrifuging Sea Urchin Eggs before and during Cleavage.”
Miss Jeanne F. Manery and Dr. Laurence Irving	“Water Changes in Trout Eggs at the Time of Laying.”
Dr. L. G. Barth	‘Chemical Nature of the Amphibian Organizer.”
Dr. John Runnström	‘Some Contributions to the Physiology of Determination in the Sea Urchin Development.”
Tuesday, July 17	
Mr. Henry I. Kohn	“The Chlorophyl Unit in Photosynthesis.”
Dr. Eric G. Ball	‘A Potentiometric Study of Phthiocol, the Pigment of the Human Tubercle Bacillus.”
Dr. S. E. Pond	“Alteration in Calcified Tissues during Drying and their Significance.”
Dr. Arthur K. Parpart	“Solvent Water in the Erythrocyte.”
Dr. William R. Duryee	‘The Relationship between the Oxygen Consumption and Water Content of the Organism.”
Tuesday, July 24	
Dr. E. Eleanor Carothers	“Spindle Fiber Attachments:—Do they Change without Chromosomal Inversions or Translocations?”
Mr. Roberts Rugh	“Ovulation and Egg Transport in the Frog.”
Dr. John W. Everett	“Certain Unusual Cytoplasmic Elements in the Yolk-sac Epithelium of the White Rat.”
Dr. Henry J. Fry	“Notes on the Behavior of Asters.”
Tuesday, July 31	
Dr. Coleen Fowler	“Permeability of Amœba proteus to Water.”
Dr. J. F. Danielli	‘The Tension at the Surface of Mackerel Oil.”
Dr. H. Burr Steinbach	“Injury Potentials in Scallop Muscles.”

Dr. Charlotte Haywood,
Miss T. Stevens,
Miss H. TeWinkel and
Miss M. Schott .... "The Relative Effects of Increased Carbon Dioxide and Diminished Oxygen upon the Heart Rate of Young Trout."

Dr. Robert Chambers .... "The Hyaline Plasma Membrane of the Echinoderm Egg."

Tuesday, August 7

Dr. Victor Schechter .... "Effect of Centrifuging on the Alga, Griffithsia."

Dr. L. G. Livingston .... "Plasmodesma in Plant Tissue."

Mr. Charles E. Renn .... "Concerning the Disappearance of the Eel Grass (Zostera marina)."

Dr. Selman A. Waksman and
Dr. Cornelia L. Carey .... "Origin and Chemical Nature of Organic Matter in the Sea Water and Sea Bottom."

Tuesday, August 14

Dr. Adrian Buyse .... "The Differentiation of Rat Gonad Primordia in Normal Adult and Gonadectomized Rat Hosts."

Dr. Caswell Grave .... "The Acceleration of Metamorphosis of Ascidian Larvæ."

Dr. E. R. Clark and
Mrs. E. L. Clark .... "Observations on the Formation of Arteriovenous Anastomoses."

Dr. C. C. Speidel .... "Experimental Study of Striated Muscle in Vivo."

Tuesday, August 21

Dr. Morris H. Harnly .... "The Temperature-effective Periods and 'Growth Curves' of the Vestigial Wings of Drosophila melanogaster."

Dr. P. W. Whiting .... "Selective Fertilization in Habrobracon."

Dr. B. R. Speicher .... 'Maturation, Fertilization and Cleavage of Habrobracon Eggs as Revealed by the Feulgen Reaction."

Dr. H. H. Plough and
Mr. P. I. Ives .... "Mutations and Modifications in Drosophila Induced by Sub-lethal High Temperature."

Friday, August 24

Dr. M. H. Jacobs and
Mr. Samuel A. Corson .... "The Influence of Minute Traces of Copper on Certain Hemolytic Processes."

Dr. David R. Goddard .... "The Keratinoid Proteins and Enzyme Digestion."

Mr. C. P. Winsor .... "The Theoretical Significance of Talbot's Law on Photoreception."

Dr. George Wald .... "Carotenoids and the Vitamin A Cycle in Vision."

Friday, August 31

Dr. E. Eleanor Carothers ...... "The Chromosomal Complex of the Mexican Grasshopper, Machærocera sumichrasti."

Dr. R. L. Carpenter .... "Regeneration in Brachial Nerves of Amblystoma."

Dr. Carl Caskey Speidel .... "Polariscopic Observations of Striated Muscle of Vertebrates and Arthropods."

Dr. Paul R. Orr .... "A Preliminary Study of Heat Death in Marine Organisms."

Mr. Ralph Oesting and
Dr. W. C. Allee .... "Further Analysis of the Protective Value of Biologically Conditioned Fresh Water for the Marine Turbellarian, Procerodes."

Mr. Roberts Rugh .... "Induced Sexual Reactions in Anura of the Woods Hole Region."

Dr. W. C. Allee and
Miss Gertrude Evans .... "Effect of Numbers on Rate of Cleavage and Early Development of Arbacia."

Mr. John A. Frank .... "The Nature of a Specific Egg-agglutinin Extracted from the Sperm of Arbacia."

Dr. Eric G. Ball .... "Isolation of Crystalline Echinochrome."

Miss Catherine E. Goffin and
Dr. Samuel E. Pond .... "Rapid Drying of Wet Marine Tissues by Two Liquid Phases and a Gas Phase."

Dr. Samuel E. Pond .... "New Steps in the Quantitative Determination of Magnesium."

Dr. Dugald E. S. Brown and
Dr. Ferdinand J. M. Sichel .... "The Myogram of the Single Muscle Fibre."

Dr. Ferdinand J. M. Sichel and
Dr. Dugald E. S. Brown .... "The Contraction of Single Muscle Fibres in Relation to the Intensity and Duration of Electrical Stimuli."

Dr. Dayton J. Edwards .... "The Action of Hydrostatic Pressure on Two Components in a Striated Muscle Response."

Dr. G. Saslow and
Mr. E. C. Webster .... "The Tension Output of Caffeinized Muscles."

Dr. Walter S. Root	"The Respiratory Metabolism of Dogfish Nerve with Special Reference to Post-mortem Change."
Miss Mary B. Derrickson and Dr. William R. Amberson	"Determination of Blood Volume in the Lower Vertebrates by the Direct Method."
Dr. M. H. Jacobs and Mr. H. N. Glassman	"'Leakage' as a Factor in the Anomalous Osmotic Behavior of the Erythrocyte."
Dr. D. E. S. Brown and Dr. Douglas Marsland	"Effects of High Hydrostatic Pressure on Amœboid Movement. The Pressure Coefficient of Viscosity in Amœba protoplasm."
Dr. Douglas Marsland and Dr. D. E. S. Brown	"Amœboid Movement under Hydrostatic Pressure."
Dr. A. K. Parpart and Mrs. E. R. Parpart	"Solvent Water in the Unfertilized Egg of Arbacia."
Dr. Coleen Fowler	"Effect of the Alternating Current on the Permeability of the Sea Urchin Eggs to Water."
Dr. Dorothy R. Stewart and Dr. M. H. Jacobs	"Further Studies on the Permeability of the Arbacia Egg to Water and to Ethylene Glycol."
Dr. L. V. Beck and Dr. R. Chambers	"Penetration of Acids into Sand Dollar Eggs and Yeast Cells."
Mr. Daniel Mazia	"Changes in the Distribution of Calcium in the Arbacia Egg in Fertilization and NaCl Cytolysis."
Dr. Katsuma Dan	"The Effect of Sodium and Calcium Ions on the Surface Charge of Arbacia Eggs."
Dr. L. V. Heilbrunn and Miss R. A. Young	"Cell Hormones and X-ray Effects on Arbacia Eggs."
Dr. L. V. Heilbrunn	"The Action of Ammonium Salts on Fatty Constituents of the Arbacia Egg."
Miss Anna K. Keltch, Miss Lucille W. Wade and Dr. G. H. A. Clowes	"Contrasting Curves of Resistance Exhibited by Arbacia Eggs Exposed to Various Chemical Agents during the Cleavage Cycle."

Dr. M. E. Krahl and Dr. G. H. A. Clowes	“Action of Dinitro Compounds on Respiration and Cell Division in Arbacia Eggs.”
Dr. G. H. A. Clowes and Dr. M. E. Krahl	“Effect on Respiration and Cell Division of Dinitrocresol and Cyanide Used in Combination.”

DEMONSTRATIONS

Dr. R. L. Carpenter	“Regeneration in Brachial Nerves of Amblystoma.” A moving picture film.
Dr. S. E. Pond and Mr. L. F. Boss	“Experimental Tests of Wood for Benches, Treated to Resist Chemicals.” “Protective Treatment of Metal against Corrosion by Sea Water.” “Refrigerators and Types of Refrigeration Units which Meet Certain Laboratory Requirements.”
Dr. Eric G. Ball	“Echinochrome Crystals.”
Dr. E. R. Clark and Mrs. E. L. Clark	“Capillary Circulation in the Rabbit's Ear.”
Dr. W. R. Duryee	“Demonstration of a Microrespirometer.”
Dr. E. Eleanor Carothers	“Extreme Chromatin Diffusion in Growth Period of First Spermatocytes of Machærocera.”
Dr. S. E. Pond and Miss Catherine E. Goffin	“Determination of Magnesium as MgO, as a Step in the Comprehensive Analysis of Calcified Tissues. a. Colored Porcelain Crucibles as a New Tool for Quantitative Analytical Chemistry.” “Drying of Marine and Other Tissues, Blood, etc., by Means of Two Liquid Phases (Benzol: Water) in Moderately Low Pressures. a. Simple and Rapid Drying Technique. Centrifugation, Milling, Benzol and Hydrogen Drying.”

7. MEMBERS OF THE CORPORATION

1. Life Members

Allis, Mr. E. P., Jr., Palais Carnoles, Menton, France.
Andrews, Mrs. Gwendolen Foulke, Baltimore, Md.
Billings, Mr. R. C., 66 Franklin St., Boston, Mass.
Conklin, Prof. Edwin G., Princeton University, Princeton, N. J.
Coolidge, Mr. C. A., Ames Building, Boston, Mass.
Crane, Mr. C. R., New York City.
Evans, Mrs. Glendower, 12 Otis Place, Boston, Mass.
Fay, Miss S. B., 88 Mt. Vernon St., Boston, Mass.
Foot, Miss Katherine, Care of Morgan Harjes Cie, Paris, France.
Gardiner, Mrs. E. G., Woods Hole, Mass.
Jackson, Miss M. C., 88 Marlboro St., Boston, Mass.
Jackson, Mr. Chas. C., 24 Congress St., Boston, Mass.
Kidder, Mr. Nathaniel T., Milton, Mass.
King, Mr. Chas. A.
Lee, Mrs. Frederic S., 279 Madison Ave., New York City, N. Y.
Lowell, Mr. A. Lawrence, 17 Quincy St., Cambridge, Mass.
McMurrich, Prof. J. P., University of Toronto, Toronto, Canada.
Means, Dr. James Howard, 15 Chestnut St., Boston, Mass.
Merriman, Mrs. Daniel, 73 Bay State Road, Boston, Mass.
Minns, Miss Susan, 14 Louisburg Square, Boston, Mass.
Morgan, Mr. J. Pierpont, Jr., Wall and Broad Sts., New York City, N. Y.
Morgan, Prof. T. H., Director of Biological Laboratory, California Institute of Technology, Pasadena, Calif.
Morgan, Mrs. T. H., Pasadena, Calif.
Noyes, Miss Eva J.
Osborn, Prof. Henry F., American Museum of Natural History, New York, N. Y.
Phillips, Mrs. John C., Windy Knob, Wenham, Mass.
Porter, Dr. H. C., University of Pennsylvania, Philadelphia, Pa.
Sears, Dr. Henry F., 86 Beacon St., Boston, Mass.
Shedd, Mr. E. A.
Thorndike, Dr. Edward L., Teachers College, Columbia University, New York City, N. Y.
Trelease, Prof. William, University of Illinois, Urbana, Ill.
Ware, Miss Mary L., 41 Brimmer St., Boston, Mass.
Wilson, Dr. E. B., Columbia University, New York City, N. Y.

2. Regular Members, 1934

Adams, Dr. A. Elizabeth, Mount Holyoke College, South Hadley, Mass.
Addison, Dr. W. H. F., University of Pennsylvania Medical School, Philadelphia, Pa.
Adolph, Dr. Edward F., University of Rochester, School of Medicine and Dentistry, Rochester, N. Y.
Allee, Dr. W. C., The University of Chicago, Chicago, Ill.
Allyn, Dr. Harriet M., Mount Holyoke College, South Hadley, Mass.
Amberson, Dr. William R., University of Tennessee, Memphis, Tenn.
Anderson, Dr. E. G., California Institute of Technology, Pasadena, Calif.
Armstrong, Dr. Philip B., Cornell University Medical College, 1300 York Avenue, New York City, N. Y.
Austin, Dr. Mary L., Wellesley College, Wellesley, Mass.
Baitsell, Dr. George A., Yale University, New Haven, Conn.
Baldwin, Dr. F. M., University of Southern California, Los Angeles, Calif.
Ball, Dr. Eric G., Johns Hopkins Medical School, Baltimore, Md.
Bard, Prof. Philip, Johns Hopkins Medical School, Baltimore, Md.
Barron, Dr. E. S. Guzman, Department of Medicine, The University of Chicago, Chicago, Ill.
Barth, Dr. L. G., Columbia University, New York City, N. Y.
Beckwith, Dr. Cora J., Vassar College, Poughkeepsie, N. Y.
Behre, Dr. Elinor H., Louisiana State University, Baton Rouge, La.
Bennitt, Dr. Rudolf, University of Missouri, Columbia, Mo.
Bigelow, Dr. H. B., Museum of Comparative Zoölogy, Cambridge, Mass.
Bigelow, Prof. R. P., Massachusetts Institute of Technology, Cambridge, Mass.
Binford, Prof. Raymond, Guilford College, Guilford College, N. C.
Bissonnette, Dr. T. H., Trinity College, Hartford, Conn.
Blanchard, Prof. Kenneth C., New York University, Washington Square College, New York City, N. Y.
Bodine, Dr. J. H., University of Iowa, Iowa City, Ia.
Boring, Dr. Alice M., Yenching University, Peking, China.
Bowling, Miss Rachel, Columbia University, New York City, N. Y.
Box, Miss Cora M., University of Cincinnati, Cincinnati, O.
Bradley, Prof. Harold C., University of Wisconsin, Madison, Wis.
Bridges, Dr. Calvin B., California Institute of Technology, Pasadena, Calif.

Bronk, Dr. D. W., University of Pennsylvania, Philadelphia, Pa.
Brooks, Dr. S. C., University of California, Berkeley, Calif.
Buckingham, Miss Edith N., Sudbury, Mass.
Budington, Prof. R. A., Oberlin College, Oberlin, O.
Bullington, Dr. W. E., Randolph-Macon College, Ashland, Va.
Bumpus, Prof. H. C., Duxbury, Mass.
Byrnes, Dr. Esther E., 1803 North Camac Street, Philadelphia, Pa.
Calkins, Prof. Gary N., Columbia University, New York City, N. Y.
Calvert, Prof. Philip P., University of Pennsylvania, Philadelphia, Pa.
Cannan, Prof. R. K., University and Bellevue Hospital Medical College, New York City, N. Y.
Carlson, Prof. A. J., The University of Chicago, Chicago, Ill.
Carothers, Dr. E. Eleanor, University of Pennsylvania, Philadelphia, Pa.
Carpenter, Dr. Russell L., College of Physicians and Surgeons, Columbia University, 630 West 168th Street, New York City, N. Y.
Carroll, Prof. Mitchel, Franklin and Marshall College, Lancaster, Pa.
Carver, Prof. Gail L., Mercer University, Macon, Ga.
Cattell, Dr. McKeen, Cornell University Medical College, 1300 York Avenue, New York City, N. Y.
Cattell, Prof. J. McKeen, Garrison-on-Hudson, N. Y.
Cattell, Mr. Ware, Garrison-on-Hudson, N. Y.
Chambers, Dr. Robert, Washington Square College, New York University, Washington Square, New York City, N. Y.
Cheney, Dr. Ralph H., Biology Department, Long Island University, Brooklyn, N. Y.
Chidester, Prof. F. E., University of Michigan, Ann Arbor, Mich.
Child, Prof. C. M., The University of Chicago, Chicago, Ill.
Clapp, Prof. Cornelia M., Montague, Mass.
Clark, Prof. E. R., University of Pennsylvania, Philadelphia, Pa.
Cleland, Prof. Ralph E., Goucher College, Baltimore, Md.
Clowes, Prof. G. H. A., Eli Lilly and Co., Indianapolis, Ind.
Coe, Prof. W. R., Yale University, New Haven, Conn.
Cohn, Dr. Edwin J., 183 Brattle Street, Cambridge, Mass.
Cole, Dr. Elbert C., Williams College, Williamstown, Mass.
Cole, Dr. Kenneth S., College of Physicians and Surgeons, 630 West 168th Street, New York City, N. Y.
Cole, Dr. Leon J., College of Agriculture, Madison, Wis.
Collett, Dr. Mary E., Western Reserve University, Cleveland, O.
Colton, Prof. H. S., Box 127, Flagstaff, Ariz.

Coonfield, Dr. B. R., Brooklyn College, 80 Willoughby Street, Brooklyn, N. Y.

Copeland, Prof. Manton, Bowdoin College, Brunswick, Me.

Costello, Dr. Donald P., Hopkins Marine Station, Pacific Grove, Calif.

Cowdry, Dr. E. V., Washington University, St. Louis, Mo.

Crampton, Prof. H. E., Barnard College, Columbia University, New York City, N. Y.

Crane, Mrs. C. R., Woods Hole, Mass.

Curtis, Dr. Maynie R., Crocker Laboratory, Columbia University, New York City, N. Y.

Curtis, Prof. W. C., University of Missouri, Columbia, Mo.

Davis, Dr. Donald W., College of William and Mary, Williamsburg, Va.

Dawson, Dr. A. B., Harvard University, Cambridge, Mass.

Dawson, Dr. J. A., The College of the City of New York, New York City, N. Y.

Dederer, Dr. Pauline H., Connecticut College, New London, Conn.

Dodds, Prof. G. S., Medical School, University of West Virginia, Morgantown, W. Va.

Dolley, Prof. William L., University of Buffalo, Buffalo, N. Y.

Donaldson, Prof. H. H., Wistar Institute of Anatomy and Biology, Philadelphia, Pa.

Donaldson, Dr. John C., University of Pittsburgh, School of Medicine, Pittsburgh, Pa.

Drew, Prof. Gilman A., Eagle Lake, Fla.

DuBois, Dr. Eugene F., Cornell University Medical College, 1300 York Avenue, New York City, N. Y.

Duggar, Dr. Benjamin M., University of Wisconsin, Madison, Wis.

Dungay, Dr. Neil S., Carleton College, Northfield, Minn.

Duryee, Dr. William R., Department of Zoölogy, Northwestern University, Evanston, Ill.

Edwards, Dr. D. J., Cornell University Medical College, 1300 York Avenue, New York City, N. Y.

Ellis, Dr. F. W., Monson, Mass.

Farnum, Dr. Louise W., Hsiang-Ya Hospital, Changsha, Hunan, China.

Fauré-Frémiet, Prof. Emmanual, Collège de France, Paris, France.

Fleisher, Dr. Moyer S., St. Louis University, School of Medicine, St. Louis, Mo.

Forbes, Dr. Alexander, Harvard University Medical School, Boston, Mass.

Fry, Dr. Henry J., Cornell University Medical College, 1300 York Avenue, New York City, N. Y.

Gage, Prof. S. H., Cornell University, Ithaca, N. Y.

Galtsoff, Dr. Paul S., 2707 Adams Mill Road, Washington, D. C.

Garrey, Prof. W. E., Vanderbilt University Medical School, Nashville, Tenn.

Gates, Prof. R. Ruggles, University of London, London, England.

Geiser, Dr. S. W., Southern Methodist University, Dallas, Tex.

Gerard, Prof. R. W., The University of Chicago, Chicago, Ill.

Glaser, Prof. O. C., Amherst College, Amherst, Mass.

Goldforb, Prof. A. J., College of the City of New York, New York City, N. Y.

Goodrich, Prof. H. B., Wesleyan University, Middletown, Conn.

Graham, Dr. J. Y., University of Alabama, University, Ala.

Grave, Prof. B. H., DePauw University, Greencastle, Ind.

Grave, Prof. Caswell, Washington University, St. Louis, Mo.

Gray, Prof. Irving E., Duke University, Durham, N. C.

Greenman, Prof. M. J., Wistar Institute of Anatomy and Biology, Philadelphia, Pa.

Gregory, Dr. Louise H., Barnard College, Columbia University, New York City, N. Y.

Guthrie, Dr. Mary J., University of Missouri, Columbia, Mo.

Guyer, Prof. M. F., University of Wisconsin, Madison, Wis.

Hadley, Charles E., Teachers' College, Montclair, N. J.

Hague, Dr. Florence, Sweet Briar College, Sweet Briar, Va.

Hall, Prof. Frank G., Duke University, Durham, N. C.

Hance, Dr. Robert T., University of Pittsburgh, Pittsburgh, Pa.

Hargitt, Prof. George T., Duke University, Durham, N. C.

Harman, Dr. Mary T., Kansas State Agricultural College, Manhattan, Kans.

Harnly, Dr. Morris H., New York University, Washington Square College, New York City, N. Y.

Harper, Prof. R. A., Columbia University, New York City, N. Y.

Harrison, Prof. Ross G., Yale University, New Haven, Conn.

Harvey, Mrs. E. N., Princeton, N. J.

Harvey, Prof. E. N., Princeton University, Princeton, N. J.

Hayden, Dr. Margaret A., Wellesley College, Wellesley, Mass.

Hayes, Dr. Frederick R., Zoölogical Laboratory, Dalhousie University, Halifax, Nova Scotia.

Haywood, Dr. Charlotte, Mount Holyoke College, South Hadley, Mass.

Hazen, Dr. T. E., Barnard College, Columbia University, New York City, N. Y.

Hecht, Dr. Selig, Columbia University, New York City, N. Y.
Heilbrunn, Dr. L. V., University of Pennsylvania, Philadelphia, Pa.
Henshaw, Dr. Paul S., Memorial Hospital, 2 West 106th Street, New York City, N. Y.
Hess, Prof. Walter N., Hamilton College, Clinton, N. Y.
Hill, Dr. Samuel E., The Rockefeller Institute, 66th Street and York Avenue, New York City, N. Y.
Hisaw, Dr. F. L., University of Wisconsin, Madison, Wis.
Hoadley, Dr. Leigh, Harvard University, Cambridge, Mass.
Hogue, Dr. Mary J., 503 N. High Street, West Chester, Pa.
Hooker, Prof. Davenport, University of Pittsburgh, Pittsburgh, Pa.
Hopkins, Dr. Hoyt S., New York University, College of Dentistry, New York City, N. Y.
Howe, Dr. H. E., 2702 36th Street, N. W., Washington, D. C.
Howland, Dr. Ruth B., Washington Square College, New York University, Washington Square, East, New York City, N. Y.
Hoyt, Dr. William D., Washington and Lee University, Lexington, Va.
Hyman, Dr. Libbie H., 41 West 70th Street, New York City, N. Y.
Irving, Prof. Laurence, University of Toronto, Toronto, Canada.
Jackson, Prof. C. M., University of Minnesota, Minneapolis, Minn.
Jacobs, Prof. Merkel H., University of Pennsylvania, Philadelphia, Pa.
Jenkins, Dr. George B., George Washington University, 1335 M Street, N. W., Washington, D. C.
Jennings, Prof. H. S., Johns Hopkins University, Baltimore, Md.
Jewett, Prof. J. R., Harvard University, Cambridge, Mass.
Johlin, Dr. J. M., Vanderbilt University Medical School, Nashville, Tenn.
Jordan, Prof. E. O., The University of Chicago, Chicago, Ill.
Just, Prof. E. E., Howard University, Washington, D. C.
Kaufmann, Prof. B. P., University of Alabama, University, Ala.
Keefe, Rev. Anselm M., St. Norbert College, West Depere, Wis.
Kidder, Dr. George W., Department of Biology, College of the City of New York, New York City, N. Y.
Kindred, Dr. J. E., University of Virginia, Charlottesville, Va.
King, Dr. Helen D., Wistar Institute of Anatomy and Biology, Philadelphia, Pa.
King, Dr. Robert L., State University of Iowa, Iowa City, Ia.
Kingsbury, Prof. B. F., Cornell University, Ithaca, N. Y.
Knapke, Rev. Bede, St. Bernard's College, St. Bernard, Ala.
Knower, Prof. H. McE., Osborn Zoölogical Laboratory, Yale University, New Haven, Conn.

Knowlton, Prof. F. P., Syracuse University, Syracuse, N. Y.

Kostir, Dr. W. J., Ohio State University, Columbus, O.

Kribs, Dr. Herbert, 250 Copley Road, Upper Darby, Pa.

Lancefield, Dr. D. E., Columbia University, New York City, N. Y.

Lange, Dr. Mathilde M., Wheaton College, Norton, Mass.

Lee, Prof. F. S., College of Physicians and Surgeons, New York City, N. Y.

Lewis, Prof. I. F., University of Virginia, Charlottesville, Va.

Lewis, Prof. W. H., Johns Hopkins University, Baltimore, Md.

Lillie, Prof. Frank R., The University of Chicago, Chicago, Ill.

Lillie, Prof. Ralph S., The University of Chicago, Chicago, Ill.

Linton, Prof. Edwin, University of Pennsylvania, Philadelphia, Pa.

Loeb, Prof. Leo, Washington University Medical School, St. Louis, Mo.

Lowther, Mrs. Florence DeL., Barnard College, Columbia University, New York City, N. Y.

Lucke, Prof. Balduin, University of Pennsylvania, Philadelphia, Pa.

Luscombe, Mr. W. O., Woods Hole, Mass.

Lynch, Dr. Clara J., Rockefeller Institute, New York City, N. Y.

Lynch, Dr. Ruth Stocking, Johns Hopkins University, Baltimore, Md.

MacDougall, Dr. Mary S., Agnes Scott College, Decatur, Ga.

McClung, Prof. C. E., University of Pennsylvania, Philadelphia, Pa.

McGregor, Dr. J. H., Columbia University, New York City, N. Y.

Macklin, Dr. Charles C., School of Medicine, University of Western Ontario, London, Canada.

Malone, Prof. E. F., University of Cincinnati, Cincinnati, O.

Manwell, Dr. Reginald D., Syracuse University, Syracuse, N. Y.

Martin, Prof. E. A., Department of Biology, Brooklyn College, 80 Willoughby Street, Brooklyn, N. Y.

Mast, Prof. S. O., Johns Hopkins University, Baltimore, Md.

Mathews, Prof. A. P., University of Cincinnati, Cincinnati, O.

Matthews, Dr. Samuel A., Department of Anatomy, University of Pennsylvania, Philadelphia, Pa.

Mavor, Prof. James W., Union College, Schenectady, N. Y.

Medes, Dr. Grace, Lankenau Research Institute, Philadelphia, Pa.

Meigs, Dr. E. B., Dairy Division Experiment Station, Beltsville, Md.

Meigs, Mrs. E. B., 1736 M Street, N. W., Washington, D. C.

Metcalf, Prof. M. M., 94 Nehoiden Road, Waban, Mass.

Metz, Prof. Charles W., Johns Hopkins University, Baltimore, Md.

Michaelis, Dr. Leonor, Rockefeller Institute, New York City, N. Y.

Miller, Dr. Helen M., Washington University, St. Louis, Mo.

Mitchell, Dr. Philip H., Brown University, Providence, R. I.
Moore, Dr. Carl R., The University of Chicago, Chicago, Ill.
Moore, Prof. George T., Missouri Botanical Garden, St. Louis, Mo.
Moore, Prof. J. Percy, University of Pennsylvania, Philadelphia, Pa.
Morgulis, Dr. Sergius, University of Nebraska, Lincoln, Nebr.
Morrill, Prof. C. V., Cornell University Medical College, New York City, N. Y.
Neal, Prof. H. V., Tufts College, Tufts College, Mass.
Nelsen, Olin E., Department of Zoölogy, University of Pennsylvania, Philadelphia, Pa.
Newman, Prof. H. H., The University of Chicago, Chicago, Ill.
Nichols, Dr. M. Louise, Rosemont, Pa.
Noble, Dr. Gladwyn K., American Museum of Natural History, New York City, N. Y.
Nonidez, Dr. Jose F., Cornell University Medical College, 1300 York Avenue, New York City, N. Y.
Okkelberg, Dr. Peter, University of Michigan, Ann Arbor, Mich.
Osburn, Prof. R. C., Ohio State University, Columbus, O.
Osterhout, Mrs. W. J. V., Rockefeller Institute, New York City, N. Y.
Osterhout, Prof. W. J. V., Rockefeller Institute, New York City, N. Y.
Packard, Dr. Charles, Columbia University, Institute of Cancer Research, 1145 Amsterdam Ave., New York City, N. Y.
Page, Dr. Irvine H., Rockefeller Institute, New York City, N. Y.
Pappenheimer, Dr. A. M., Columbia University, New York City, N. Y.
Parker, Prof. G. H., Harvard University, Cambridge, Mass.
Patten, Dr. Bradley M., Rockefeller Foundation, 49 West 49th Street, New York City, N. Y.
Payne, Prof. F., University of Indiana, Bloomington, Ind.
Pearl, Prof. Raymond, Institute for Biological Research, 1901 East Madison Street, Baltimore, Md.
Peebles, Prof. Florence, California Christian College, Los Angeles, Calif.
Phillips, Dr. E. F., Cornell University, Ithaca, N. Y.
Pinney, Dr. Mary E., Milwaukee-Downer College, Milwaukee, Wis.
Plough, Prof. Harold H., Amherst College, Amherst, Mass.
Pollister, Dr. A. W., Columbia University, New York City, N. Y.
Pond, Dr. Samuel E., Marine Biological Laboratory, Woods Hole, Mass.
Pratt, Dr. Frederick H., Boston University, School of Medicine, Boston, Mass.

Raffel, Dr. Daniel, Zoölogical Laboratory, The Johns Hopkins University, Baltimore, Md.

Rand, Dr. Herbert W., Harvard University, Cambridge, Mass.

Redfield, Dr. Alfred C., Harvard University, Cambridge, Mass.

Reese, Prof. Albert M., West Virginia University, Morgantown, W. Va.

de Renyi, Dr. George S., Department of Anatomy, University of Pennsylvania, Philadelphia, Pa.

Reznikoff, Dr. Paul, Cornell University Medical College, 1300 York Avenue, New York City, N. Y.

Rice, Prof. Edward L., Ohio Wesleyan University, Delaware, O.

Richards, Prof. A., University of Oklahoma, Norman, Okla.

Richards, Dr. O. W., Osborn Zoölogical Laboratory, Yale University, New Haven, Conn.

Riggs, Mr. Lawrason, Jr., 120 Broadway, New York City, N. Y.

Rogers, Prof. Charles G., Oberlin College, Oberlin, O.

Romer, Dr. Alfred S., The University of Chicago, Chicago, Ill.

Root, Dr. W. S., Syracuse Medical School, Syracuse, N. Y.

Sayles, Dr. Leonard P., Department of Biology, College of the City of New York, 139th Street and Convent Avenue, New York City, N. Y.

Schotté, Dr. Oscar E., Department of Biology, Amherst College, Amherst, Mass.

Schrader, Dr. Franz, Department of Zoölogy, Columbia University, New York City, N. Y.

Schrader, Dr. Sally Hughes, Department of Zoölogy, Columbia University, New York City, N. Y.

Schramm, Prof. J. R., University of Pennsylvania, Philadelphia, Pa.

Scott, Dr. Ernest L., Columbia University, New York City, N. Y.

Scott, Prof. G. G., College of the City of New York, New York City, N. Y.

Scott, Prof. John W., University of Wyoming, Laramie, Wyo.

Scott, Prof. William B., 7 Cleveland Lane, Princeton, N. J.

Severinghaus, Dr. Aura E., Department of Anatomy, College of Physicians and Surgeons, 630 W. 168th Street, New York City, N. Y.

Shull, Prof. A. Franklin, University of Michigan, Ann Arbor Mich.

Shumway, Dr. Waldo, University of Illinois, Urbana, Ill.

Sichel, Dr. Ferdinand J. M., Department of Zoölogy, University of Pennsylvania, Philadelphia, Pa.

Sivickis, Dr. P. B., Pasto deze 130, Kaunas, Lithuania.

Snow, Dr. Laetitia M., Wellesley College, Wellesley, Mass.
Sollman, Dr. Torald, Western Reserve University, Cleveland, O.
Sonneborn, Dr. T. M., Johns Hopkins University, Baltimore, Md.
Speidel, Dr. Carl C., University of Virginia, University, Va.
Stabler, Dr. Robert M., Department of Zoölogy, University of Pennsylvania, Philadelphia, Pa.
Stark, Dr. Mary B., New York Homeopathic Medical College and Flower Hospital, New York City, N. Y.
Steinbach, Dr. Henry B., University of Rochester, Rochester, N. Y.
Stewart, Dr. Dorothy R., Skidmore College, Saratoga Springs, N. Y.
Stockard, Prof. C. R., Cornell University Medical College, 1300 York Avenue, New York City, N. Y.
Stokey, Dr. Alma G., Mount Holyoke College, South Hadley, Mass.
Strong, Prof. O. S., College of Physicians and Surgeons, 630 West 168th Street, New York City, N. Y.
Stunkard, Dr. Horace W., New York University, University Heights, N. Y.
Sturtevant, Dr. Alfred H., California Institute of Technology, Pasadena, Calif.
Sumwalt, Dr. Margaret, 2901 Chelsea Terrace, Baltimore, Md.
Swett, Dr. Francis H., Duke University Medical School, Durham, N. C.
Taft, Dr. Charles H., Jr., University of Texas Medical School, Galveston, Tex.
Tashiro, Dr. Shiro, Medical College, University of Cincinnati, Cincinnati, O.
Taylor, Dr. William R., University of Michigan, Ann Arbor, Mich.
Tennent, Prof. D. H., Bryn Mawr College, Bryn Mawr, Pa.
Thatcher, Mr. Lloyd E., Spring Hill, Tenn.
Tracy, Prof. Henry C., University of Kansas, Lawrence, Kans.
Treadwell, Prof. A. L., Vassar College, Poughkeepsie, N. Y.
Turner, Prof. C. L., Northwestern University, Evanston, Ill.
Tyler, Dr. Albert, California Institute of Technology, Pasadena, Calif.
Uhlenhuth, Dr. Eduard, University of Maryland, School of Medicine, Baltimore, Md.
Unger, Dr. W. Byers, Dartmouth College, Hanover, N. H.
Van der Heyde, Dr. H. C., Galeria, Corse, France.
Visscher, Dr. J. Paul, Western Reserve University, Cleveland, O.
Waite, Prof. F. C., Western Reserve University Medical School, Cleveland, O.
Wallace, Dr. Louise B., 660 Waverley Street, Palo Alto, Calif.
Ward, Prof. Henry B., University of Illinois, Urbana, Ill.

WARREN, DR. HERBERT S., Department of Biology, Temple University, Philadelphia, Pa.
WATERMAN, ALLYN J., Department of Biology, Williams College, Williamstown, Mass.
WENRICH, DR. D. H., University of Pennsylvania, Philadelphia, Pa.
WHEDON, DR. A. D., North Dakota Agricultural College, Fargo, N. D.
WHEELER, PROF. W. M., Museum of Comparative Zoölogy, Cambridge, Mass.
WHERRY, DR. W. B., Cincinnati Hospital, Cincinnati, O.
WHITAKER, DR. DOUGLAS M., P. O. Box 2514, Stanford University, Calif.
WHITE, DR. E. GRACE, Wilson College, Chambersburg, Pa.
WHITING, DR. PHINEAS W., Carnegie Station, Cold Spring Harbor, Long Island, N. Y.
WHITNEY, DR. DAVID D., University of Nebraska, Lincoln, Nebr.
WIEMAN, PROF. H. L., University of Cincinnati, Cincinnati, O.
WILLIER, DR. B. H., Department of Zoölogy, University of Rochester, Rochester, N. Y.
WILSON, PROF. H. V., University of North Carolina, Chapel Hill, N. C.
WILSON, DR. J. W., Brown University, Providence, R. I.
WITSCHI, PROF. EMIL, University of Iowa, Iowa City, Ia.
WOGLOM, PROF. WILLIAM H., Columbia University, New York City, N. Y.
WOODRUFF, PROF. L. L., Yale University, New Haven, Conn.
WOODS, DR. FARRIS H., Lefevre Hall, Columbia, Mo.
WOODWARD, DR. ALVALYN E., Zoölogy Department, University of Michigan, Ann Arbor, Mich.
YOUNG, DR. B. P., Cornell University, Ithaca, N. Y.
YOUNG, DR. D. B., 110 Del Ray Avenue, Bethesda, Md.
ZELENY, DR. CHARLES, University of Illinois, Urbana, Ill.

THE CENTRIOLES OF PSEUDOTRICHONYMPHA AND THEIR RÔLE IN MITOSIS

L. R. CLEVELAND

(*From the Department of Tropical Medicine, Harvard University Medical School, Boston, Massachusetts*)

The genus *Pseudotrichonympha* was erected by Grassi and Foà in 1911. Seven species have been described, but the descriptions, for the most part, are too incomplete to permit one to determine whether or not the organisms here considered represent new species. The species described by Cutler (1921), as previously noted (Cleveland et al, 1934), does not belong to the genus *Pseudotrichonympha.* This leaves six species in the genus, and it is impossible, without studying each of these carefully, to determine which are valid and which are not and whether the organisms used in this study represent new species or are identical with previously described ones. The material used comes from three species of *Coptotermes,—C. curvignatus, C. travians,* and *C. ceylonicus,* and was collected and prepared for study by Miss Jane Collier. The first two species are from Java and were identified by Kalshoven; the other is from Ceylon and was identified by Jepson.

The present account is limited to a description of the structure and function of those organelles which, in all probability, are identical in all species of the genus. For a description of the relation of *Pseudotrichonympha* to other genera of the Eucomonymphidæ the reader is referred to the recent memoir by Cleveland, Hall, Sanders, and Collier (1934).

The body of 15 individuals of the *Pseudotrichonympha* in *C. curvignatus* varied in length from 200 to 458 microns and in width from 73 to 183 microns; in *C. travians* the length varied from 159 to 366 and the width from 43 to 122; and in *C. ceylonicus* the length varied from 153 to 305 and the width from 37 to 104. The entire surface is covered with flagella arranged in longitudinal rows (Fig. 15). A rostral tube, more prominent than that of *Trichonympha* but less prominent than that of *Eucomonympha* and *Teratonympha,* is present in the anterior end (Figs. 1, 12, 13). The rows of flagella line the outer surface of this tube for most of its length, leaving it near its posterior end, extending toward the periphery and continuing posteriorly to the end of the body. Those flagella arising from the rostral portion of the body are slightly longer than those arising from the post-rostral portion

(Fig. 15). The anterior end of the rostral tube is covered with an inner cap (Fig. 1) and a large outer cap covers the anterior end of the rostrum (Figs. 1–4). The rostral tube is composed of two half-cone-shaped portions which in the interphase lie close together but which move apart during cell division (Figs. 1–4). Each portion ends anteriorly in a semicircular ring which is slightly enlarged at the ends. This ring is composed of many close-fitting basal granules from which the first circulet of flagella arises and is the end of the lamella-portion of the rostral tube. Whether it is composed of anything else or not has not been determined.

The elongate centrioles extend from this ring posteriorly along the inner margin of the rostral tube to which they adhere so closely that it is frequently impossible to distinguish them from the rostral tube until they extend beyond it posteriorly (Figs. 1–4). As the two halves of the rostrum separate during division, one centriole adheres to each half, and, unlike other hypermastigotes studied (Cleveland et al, 1934) except *Eucomonympha*, no new centrioles grow out from the proximal ends of the old or functioning centrioles at this time. Each functioning centriole begins to degenerate soon after cytoplasmic division, the degeneration continuing until only the proximal portion remains, so that even though the centrioles are very long during mitosis they are short in the interphase (Figs. 11–13). As soon as the connection between the centriole and the nucleus is lost, the centriole, which at this time is very long (Fig. 11), shortens, becomes coiled (Fig. 12), and presently begins to fragment (Fig. 13). These fragments remain in the cytoplasm for some time and many stages in their disintegration have been seen. Since the centriole in the rostral region of the body is continuous with the rostral tube, it is difficult to determine whether the disintegration continues to the proximal end or stops at the base of the rostral tube.

In the very early prophase two centrioles begin to grow posteriorly from the base of the rostral tube in the direction of the nucleus, which usually lies slightly anterior to the mid-region of the body, although it occasionally lies posterior to this region. Since the two halves of the rostral tube at this stage lie practically as close together as in the interphase, the distal ends of the centrioles are also close together. These centrioles extend scarcely any distance from the rostrum before a small centrosome appears on the distal end of each. At the same time, astral rays arise from the distal end of each centriole and pass through the centrosome (Fig. 1). Presently the astral rays arising from one centriole meet those arising from the other and as they meet they join, grow along one another, and overlap to form the early central spindle (Fig.

2). Not all early central spindles have exactly the same appearance as the one illustrated by Fig. 2. They vary in breadth and length, depending on how close the proximal ends of the centrioles are together when they begin to give off astral rays. They sometimes lie so close together that the centrosomes are adjacent to each other (Fig. 1), while at other times they extend from the base of the rostral tube parallel with this organelle; and the early central spindle sometimes forms at the base of the rostral tube.

As more astral rays arise from the centrioles, more of them meet and join and the central spindle increases in size. At the same time, the centrioles become longer and broader and both their proximal and distal ends become more widely separated (Fig. 3). The proximal ends of the centrioles are moved apart by the separation of the daughter rostra and the distal ends by the elongation of the central spindle. The astral rays that have not met and overlapped are now longer than in earlier stages in the development of the central spindle, but none of them reach the nucleus, for this organelle still lies 40 or 50 microns posterior to the central spindle. However, in the next illustration the centrioles have become much longer and the central spindle is in contact with the nuclear membrane (Fig. 4). (All stages between Figs. 3 and 4 in the elongation of the centrioles, the enlargement of the central spindle, and the movement of the central spindle posteriorly toward the nucleus have been seen, but it has seemed unnecessary to illustrate them.) In the posterior elongation of the centrioles, one centriole sometimes grows faster than the other so that the central spindle does not always lie at a right angle with the long axis of the body when it first touches the nuclear membrane. In a few instances it is turned more than in the illustration (Fig. 4), while in other instances it may extend straight across the body when it first touches the nuclear membrane. Regardless of the angle of the central spindle when it touches the nuclear membrane, it soon straightens out owing to the many fibrillar connections made between the centrioles and the chromatin (Figs. 5–7). In addition to the variation of the angle of the central spindle when it reaches the nucleus, there is also considerable variation in its breadth and length. This variation is probably due to two things: the rate of growth of the centrioles and the distance between the distal ends of the centrioles when the central spindle is first formed. For example, if the distal ends of the centrioles are close together when the first astral rays join to form the central spindle and the centrioles elongate rapidly, the central spindle when it first touches the nucleus is short and narrow; on the other hand, if the ends of the centrioles are close together and the elongation of the centrioles is slow, the central spindle is broad and short; and if the posterior ends of the centrioles are a considerable distance apart when the astral rays join, the central spindle when it reaches the nucleus is long and broad if the elongation of the centrioles is slow, but if the elongation is rapid the central spindle is long and narrow. However, these irregularities in the development of the central spindle are soon ironed out after it reaches the nucleus, because the development of the achromatic figure and that of the chromosomes go hand in hand during nuclear division.

But the nucleus, which lay 40 to 60 microns away from the central spindle when it first began to develop, has not been idle during the development and posterior migration of the central spindle. The interphase connections between the chromatin and the nuclear membrane (Fig. 14) become intranuclear chromosomal fibres as the chromosomes develop and thus connect the chromosomes with the nuclear membrane. As soon as the central spindle reaches the nuclear membrane, or shortly thereafter, some of the astral rays make connection with the intranuclear chromosomal fibres and thus become extranuclear chromosomal fibres, the first such connections to be made being with the chromatin (" single spireme " stage) nearest the distal ends of the centrioles (Fig. 4). As more of these connections are made, the central spindle depresses the nuclear membrane and takes up an axial position, the chromosomes split longitudinally, the central spindle elongates, and the distal ends of the centrioles become more widely separated (Fig. 5). Presently, all the chromosomes are connected with the centrioles, half being connected with each centriole, and poleward movement of the V-shaped daughter chromosomes begins. As a matter of fact, some of the chromosomes are near the poles (centrioles) when the connections are made and remain in this position, and movement of the chromosomes that are first connected with the centrioles begins before, or at least by the time, the other connections are made, so that there is no equatorial plate stage with the chromosomes in the centre of the nucleus (Figs. 6, 7). As the distal ends of the centrioles move farther apart, the central spindle becomes longer, the nuclear membrane pulls in two, and the daughter chromosomes, which are securely fastened to the centrioles, move apart, the chromosomes becoming shorter as the process continues (Figs. 8–10). Eventually, the central spindle pulls apart, but its fibres, as well as the extranuclear chromosomal fibres, remain until the chromatin has almost returned to the interphase condition (which, as a rule, is some time after cytoplasmic division) (Fig. 11). Shortly after the disappearance of the achromatic figure, the centriole (as already described) degenerates, at least to the rostral tube, and does not elongate again until the next cell division (Figs. 12, 13, 15).

The centrioles of *Pseudotrichonympha,* in addition to being more elongate than those of any other hypermastigote previously studied (or any cell for that matter), are definitely granular and the granules appear to be embedded in a hyaline matrix in the form of a tube or strand (Fig. 4).

The centrosome which surrounds the distal end of each centriole increases in size as the centrioles elongate and the achromatic figure enlarges. The fibres of the achromatic figure do not arise from the centrosomes, but merely pass through them from the centrioles (Figs. 3–11).

The flagella and other extranuclear organelles, except the achromatic figure, arise from the proximal ends of the centrioles as described in other hypermastigotes by Cleveland, Hall, Sanders, and Collier (1934).

REFERENCES CITED

Cleveland, L. R., S. R. Hall, E. P. Sanders, and J. Collier, 1934. The Wood-feeding Roach Cryptocercus, its Protozoa, and the Symbiosis between Protozoa and Roach. *Mem. Am. Acad. Arts and Sci.,* **17**: 185.

Cutler, D. W., 1921. Observations on the Protozoa Parasitic in the Hind-gut of Archotermopsis wroughtoni Desn. Part III. Pseudotrichonympha pristina. *Quart. Jour. Micro. Sci.,* **65**: 247.

Grassi, B., and A. Foà, 1911. Intorno ai Protozoi dei Termitidi. *Rend. R. Accad. Lincei* (5), **20**: 1 sem., 725.

Explanation of Plate I

The drawings were made with the aid of a camera lucida from material fixed in Schaudinn's fluid and stained in Heidenhain's hæmatoxylin.

Fig. 1. Extreme anterior end of body (compare with Fig. 15), showing rostrum covered with outer cap, rostral tube covered anteriorly with inner cap, a small portion of body posterior to rostrum with two centrioles extending from the base of the rostral tube into the body. This is a very early prophase. The formation of the achromatic figure has just begun. Note astral rays extending from the distal end of each centriole through the centrosome. For position of nucleus at this stage, compare with Figs. 4 and 15. × 1400.

Fig. 2. Slightly later stage. The halves of the rostral tube have moved a short distance apart and some of the astral rays have joined and overlapped to form the early central spindle. Note the semicircular ring at the anterior end of each half of the rostrum. The centrioles extend from the ends of the central spindle to these semicircular rings. × 1400.

Fig. 3. Later stage, central spindle longer, centrioles longer and broader. × 1400.

Fig. 4. Central spindle has increased in size and, owing to the increase in length of the centrioles, has reached the nucleus and has begun to depress the nuclear membrane. Rostral halves more widely separated and new flagella are forming on their inner surfaces. Note granular nature of the elongate centrioles. Nucleus in "single spireme" stage. Note intranuclear chromosomal fibres connecting the developing chromosomes with the nuclear membrane. A few connections have been made between the centrioles and these intranuclear chromosomal fibres by astral rays, such rays being extranuclear chromosomal fibres. Note

increase in size of the centrosomes as compared with earlier stages in the development of the achromatic figure. × 1400.

Fig. 5. Central spindle is longer, has depressed the nuclear membrane, and is surrounded by the chromosomes which have split longitudinally. More connections have been made between the centrioles and the chromosomes, but little or no chromosomal movement has occurred. × 1400.

Fig. 6. Vertical view of a slightly later stage, omitting the chromosomes above the central spindle. Poleward movement of the chromosomes has begun and all the chromosomes are connected with the centrioles, half being connected with each centriole. × 1400.

Fig. 7. Daughter chromosomes have moved a considerable distance toward their respective poles (centrioles), central spindle and nucleus elongate, and definite intra- and extranuclear chromosomal fibres connect the large, V-shaped chromosomes with the distal ends of the centrioles. The position of the daughter rostra is indicated by the direction of the small portions of the centrioles that are illustrated (compare with Figs. 4 and 11). The enlargements on the free ends of the chromosomes are fragments of the nucleolus. × 1400.

Fig. 8. Central spindle more elongate, chromosomes at poles, and nuclear membrane almost pulled in two. × 1100.

Fig. 9. Later stage of one daughter nucleus, showing an apparent split in the chromosomes. × 1100.

Fig. 10. Central spindle is pulling apart and daughter nuclei widely separated. × 1100.

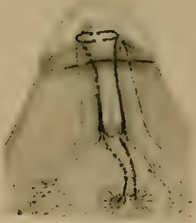
Fig. 11. After cytoplasmic division. Portion of rostrum and rostral tube shown at upper end of illustration. Note very long centriole extending from base of rostral tube to nucleus (only a portion being illustrated) and central spindle and extranuclear chromosomal fibres extending from the distal end of the centriole. Chromatin almost in interphase condition. × 1100.

Fig. 12. Rostrum, rostral tube, and central portion of anterior part of body shown. Achromatic figure has disappeared and the centriole, since it is no longer connected with nucleus, has become coiled and shorter. × 1100.

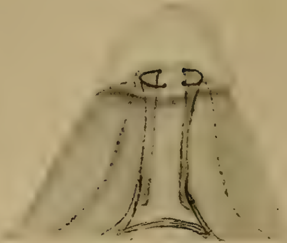
Fig. 13. Regions of body shown same as in Fig. 12. The centrosome and that portion of centriole posterior to base of rostral tube are disintegrating and will presently break into many small pieces which become scattered through the body where they are resorbed. × 1100.

Fig. 14. Interphase nucleus. Note connections between chromatin and nuclear membrane. These become the intranuclear chromosomal fibres when the chromosomes are formed. × 1100.

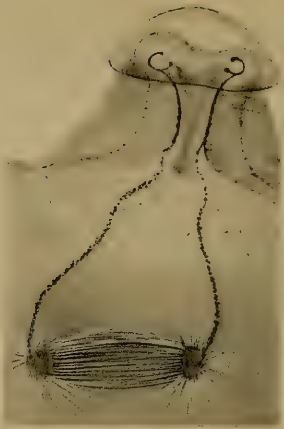
Fig. 15. Entire organism in interphase showing shape of body which is entirely covered with flagella, position of rostrum and nucleus, and many strands—either parabasals or axostyles—extending posteriorly from base of the rostrum. × 500.



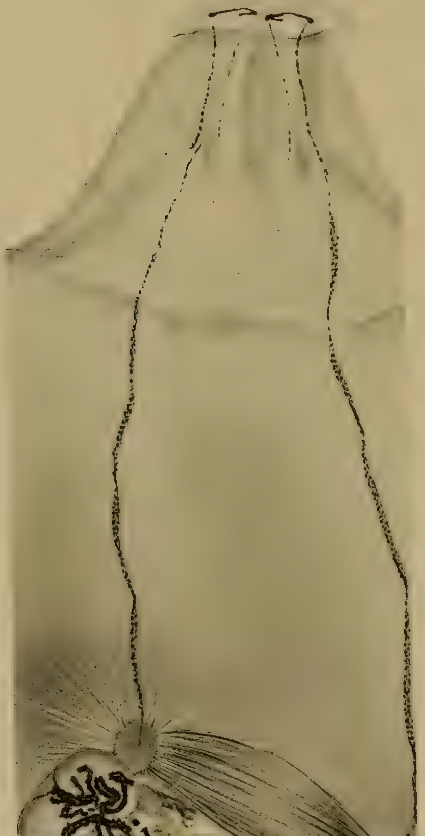
1



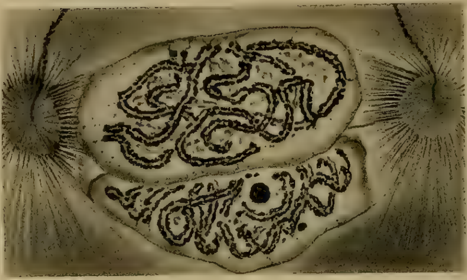
2



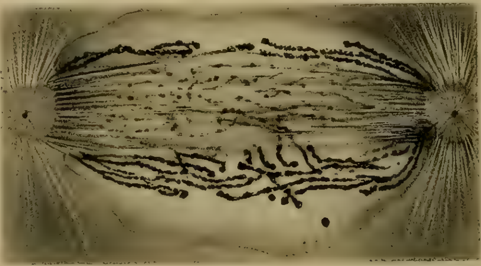
3



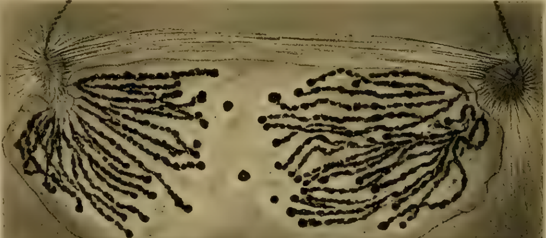
4



5



6

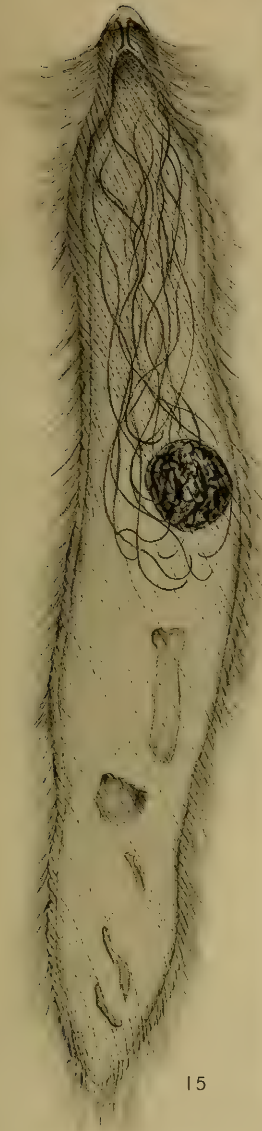


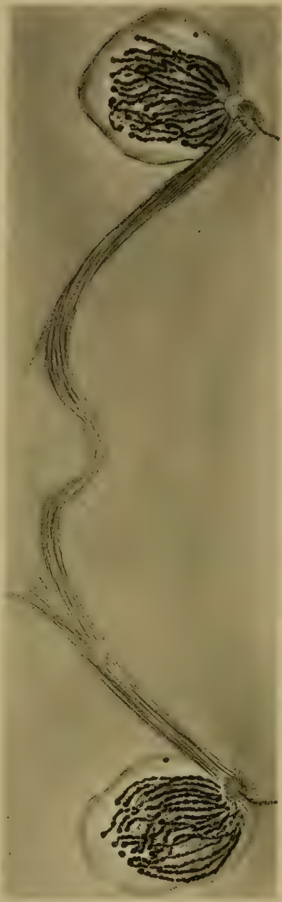
7

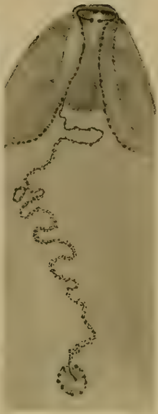
Elizabeth P. Sanders del.

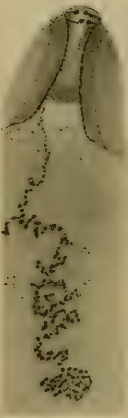
PLATE 1

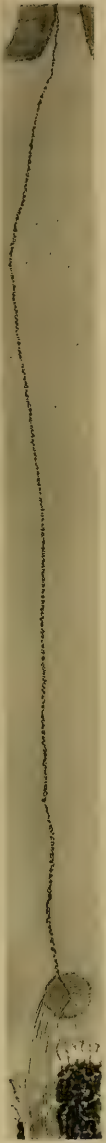
8

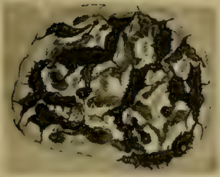

15

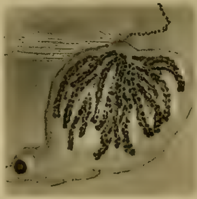

10


12


13


11


14


9

L. R. Cleveland

THE INTRANUCLEAR ACHROMATIC FIGURE OF OXYMONAS GRANDIS SP. NOV.

L. R. CLEVELAND

(*From the Department of Tropical Medicine, Harvard University Medical School, Boston, Mass.*)

Before considering the nature and development of the very large intranuclear achromatic figure of this organism, it is necessary to devote some space to a description of the other organelles since they differ considerably from those of the described species of *Oxymonas,* but perhaps not sufficiently to justify the erection of a new genus.

Morphology and Relation to Other Species

The genus *Oxymonas* was erected by Janicki in 1915 for a flagellate from *Kalotermes* (*Neotermes*) *connexus* Snyder, 1922. Fifteen other species have been described: *O. projector* Kofoid and Swezy, 1926, from *Kalotermes* (*Glyptotermes*) *perparvus* Emerson, 1925, body length 12 to 40 microns; *O. pediculosa* Kofoid and Swezy, 1926, from *Kalotermes* (*Lobitermes*) *nigriceps* Emerson, 1925, body length 27 to 40 microns; *O. gracilis* Kofoid and Swezy, 1926, from *Kalotermes* (*Rugitermes*) *magninotus* Emerson, 1925, average body length 15 microns; *O. parvula* Kirby, 1926, from *Kalotermes* (*Cryptotermes*) *hermsi* Kirby, 1925, body length 5 to 13 microns; *O. panamæ* Zeliff, 1930, from *Kalotermes* (*K.*) *panamæ* Snyder, 1924, and *K.* (*K.*) *marginipennis* Latreille, 1811, average body size 18 by 26 microns; *O. kirbyi* Zeliff, 1930, from *Kalotermes* (*Rugitermes*) *kirbyi* Snyder, 1926, average body size 16 by 26 microns; *O. clevelandi* Zeliff, 1930, from *Kalotermes* (*K.*) *clevelandi* Snyder, 1926, and *K.* (*K.*) *tabogæ* Snyder, 1924, average body size 8 by 17 microns; *O. brevis* Zeliff, 1930, from *Kalotermes* (*Cryptotermes*) *brevis* Walker, 1853, average body size 5 by 13 microns; *O. barbouri* Zeliff, 1930, from *Kalotermes* (*Glyptotermes*) *barbouri* Snyder, 1924, average body size 12 by 21 microns; *O. synderi* Zeliff, 1930, from *Kalotermes* (*Cryptotermes*) *breviarticulatus* Snyder, 1926, average body size 5 by 10 microns; *O. jouteli* Zeliff, 1930, from *Kalotermes* (*K.*) *jouteli* Banks, 1920, average body size 8 by 26 microns; *O. janicki* Zeliff, 1930, from *Kalotermes* (*Rugitermes*) *kirbyi* Snyder, 1926, average body size 8 by 18 microns; *O. ovata* Zeliff, 1930, from *Kalotermes* (*Calcaritermes*) *brevicollis* Banks, 1918, average body size 15 by 19 microns; *O. minor* Zeliff, 1930, from *Kalotermes*

(*K.*) *minor* Hagen, 1858, average body size 13 by 25 microns; *O. hubbardi* Zeliff, 1930, from *Kalotermes* (*K.*) *hubbardi* Banks, 1920, average body size 8 by 17 microns; and *O. dimorpha* Connell, 1930, from *Kalotermes* (*Paraneotermes*) *simplicicornis* (Banks), average body size in motile phase 23 by 35 microns and in attached phase 100 by 120 microns.

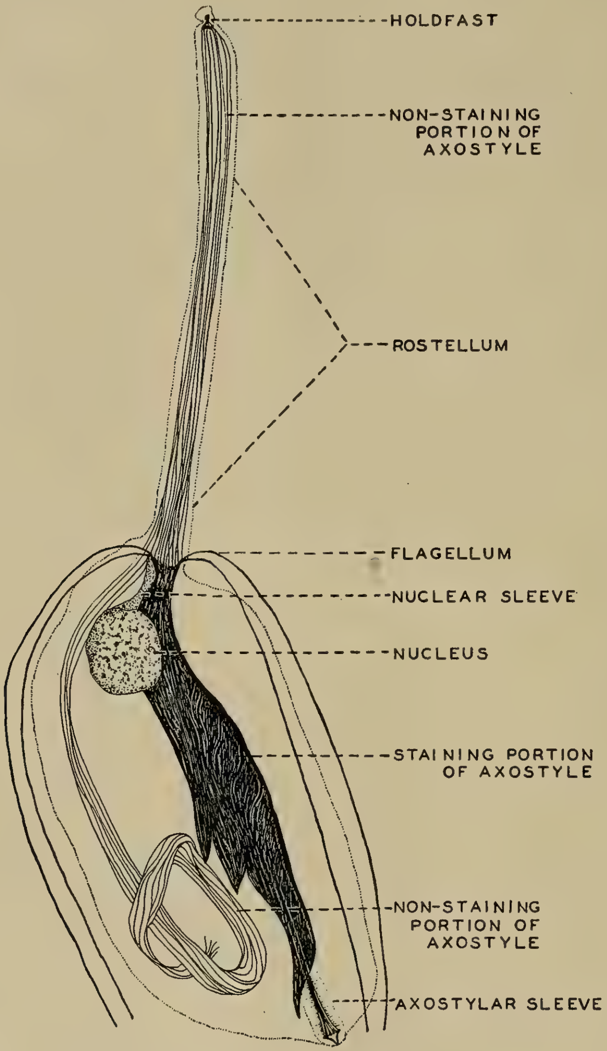
O. dimorpha is clearly a distinct species, but it is doubtful if all the other described species are valid, especially the large number described by Zeliff (1930). The fact that they occur in different species of termites does not necessarily make them valid.

Oxymonas grandis has been found so far in *Kalotermes* (*Neotermes*) *dalbergiæ* Kalshoven, 1930, and *K.* (*N.*) *tectonæ* Dammerman, 1915, and is more abundant in the material at hand in the first-named termite. A similar species, differing rather sharply in a few respects but little or not at all in other respects, occurs in *Kalotermes* (*Neotermes*) *dilatatus* Bugnion and Popoff, 1910. The first two termites, which occur in Java, were determined by Kalshoven and the other, which occurs in Ceylon, by Jepson. Miss Jane Collier (research assistant in protozoölogy) collected these termites in 1931 and prepared their protozoa for study.

The maximum body length in the species described by Kofoid and Swezy, Kirby, and Zeliff is 40 microns. Janicki gives no measurements, but from the magnification of his illustrations the body of *O. granulosa* must be approximately 10 by 20 microns. The only species that is comparable in size with *O. grandis* is *O. dimorpha* described by Connell, and it is considerably smaller than *O. grandis.* The average body width and length, exclusive of the rostellum, of fifty individuals of *O. grandis* selected at random is 52 by 121 microns, the minimum dimensions being 31 by 76 and the maximum 79 by 183 microns.

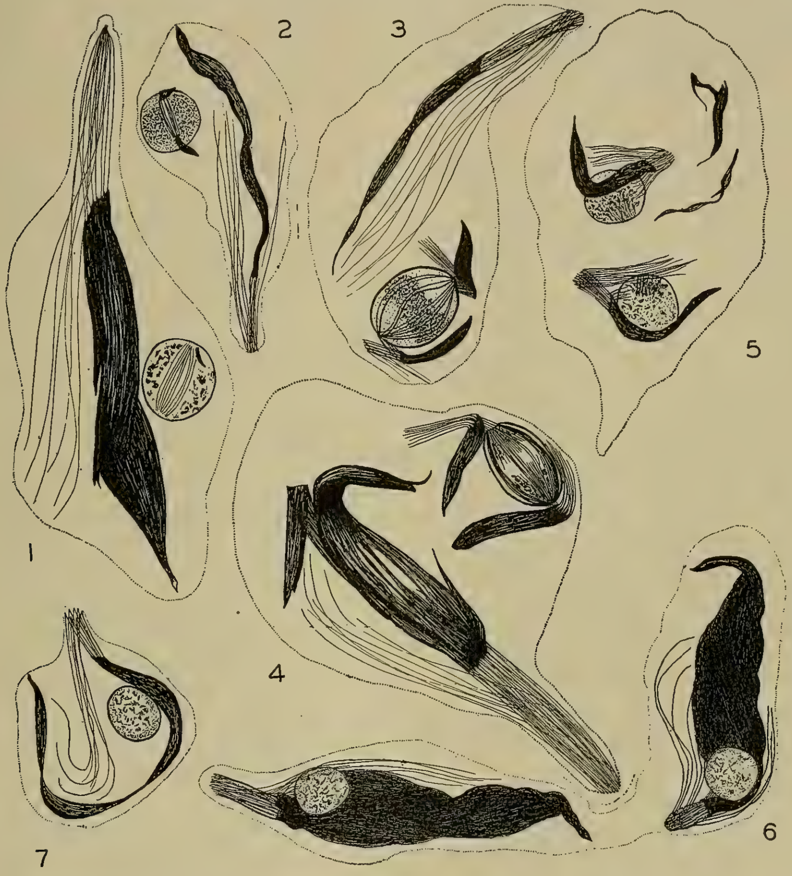
The rostellum, which is an extension of the body made by the nonstaining portion of the axostyle and which serves to anchor the organism to the chitinous intima of the hind gut, varies greatly in length and to a considerable extent in width. For example, in fifty organisms selected at random, the average length was 95 microns, the minimum 31, and the maximum 214. This, of course, does not include late telophase organisms where the axostyle is just beginning to extend the body to form the rostellum (Text-fig. *B, 7*).

The nucleus of the described species is much smaller than that of *O. grandis,* the maximum diameter in any species except *O. dimorpha* being 7 microns, and that of most species 4 microns; while the average diameter of the nucleus of twenty individuals of *O. grandis* selected at random was 21 microns, the minimum 20, and the maximum 23. The nucleus of *O. dimorpha* is only slightly larger than that of the other described species, the average diameter in ten individuals being 8 microns, the minimum 6, and the maximum 10. Thus, the nucleus of *O. grandis* is more than twice the diameter of that of any of the previously described species.


HOLDFAST
NON-STAINING PORTION OF AXOSTYLE
ROSTELLUM
FLAGELLUM
NUCLEAR SLEEVE
NUCLEUS
STAINING PORTION OF AXOSTYLE
NON-STAINING PORTION OF AXOSTYLE
AXOSTYLAR SLEEVE

Text-fig. *A*. *Oxymonas grandis*. Entire organism, showing the nature of the axostyle, position of nucleus, and attachment of nucleus to axostyle by nuclear sleeve. × 400.

O. grandis is further differentiated from the described species in the absence of a karyosome and in the presence of a broad axostyle which, at its widest portion, in ten individuals ranged from 15 to 24 microns (Text-fig. *A*). The axostyle consists of a staining and a non-staining portion, the latter beginning near the rostellum, extending into this organelle to its anterior tip where it bends and then extends posteriorly through the rostellum and the body, usually forming a coil in the posterior portion of the body, although it sometimes bends after reaching the posterior end of the body and extends anteriorly as far as the nucleus. The staining portion of the axostyle extends posteriorly from



TEXT-FIG. *B*. The connection between nucleus and axostyle is lost (1); nucleus migrates posteriorly and divides (1–5); axostyle is resorbed (1–5); a new axostyle develops from each centriole at the ends of the central spindle (1–2); at an early stage in the development of the axostyle the portion of the centriole producing the axostyle separates from that which produces the achromatic figure and migrates into the cytoplasm and most of the development of the axostyle occurs after this migration (3–7); in the late development of the axostyle, the non-staining portion extends beyond the body to produce the rostellum (7). (The flagella, which are not shown, are also discarded, resorbed, and renewed. See text.) × 300.

the rostellum through the body, the posterior portion being pointed and surrounded by an axostylar sleeve (Text-fig. *A*). The staining portion of the axostyle, instead of gradually becoming smaller as it approaches the sleeve usually frays, and in this respect differs from the broad axostyle of *Saccinobaculus;* but the non-staining portion seldom frays, and in this respect differs from that of the previously described species. Both portions of the axostyle are fibrillar and very elastic, but the fibres of the staining portion fit more closely together. As a matter of fact, this is why this portion retains the stain after the other portion loses it; in specimens too heavily stained for a study of the nucleus, both portions retain the stain, although the portion where the fibres are close together is darker than the other region and loses its fibrillar appearance altogether.

An axostyle and four flagella arise from each of the intranuclear centrioles during cell division, the old axostyle and flagella being discarded and resorbed at this time. The interphase nucleus, which is anchored to the staining portion of the axostyle by a nuclear sleeve, migrates to the posterior portion of the body before mitosis begins. In the prophase, a daughter axostyle begins to grow out from each of the circular centrioles at the ends of the central spindle. As the daughter axostyles increase in size, they move away from the nucleus and soon lose all apparent connection with the intranuclear centrioles and the dividing nucleus, but they must retain some connection with the nucleus; otherwise they would be free in the cytoplasm and it would be difficult for the daughter nuclear sleeves, as they develop from the axostylar growing points at the juxtaposition of the staining and non-staining portions of the daughter axostyles, to make connection with the daughter nuclei, surround them, and thus anchor them securely to the axostyles until the next cell division. As already indicated, and as shown in Text-fig. *B*, most of the development of the daughter axostyles occurs when there is no apparent connection between the axostyles and the centrioles from which their development begins. At an early stage in the development of each daughter axostyle there is a separation of the axostyle-producing portion of the centriole and the achromatic figure-producing portion, the latter remaining inside the nucleus and the former making its way outside and functioning in the long-continued development and growth of the axostyle. (The development of the daughter axostyles is not more than half completed when the daughter nuclei have returned to the interphase, and the axostyles continue to grow for some time after cytoplasmic division.) This extranuclear area from which the axostyle develops is situated at the line of demarcation between the staining and non-staining portions of the axostyle.

From it the staining portion of the axostyle grows posteriorly and the non-staining portion anteriorly. It is also from this area that the four flagella and the fibres composing the nuclear sleeve develop. This is an instance, then, of a separation of the achromatic figure-producing portion of the centriole from the portion that produces the extranuclear organelles. The extranuclear organelle-producing portion of the centriole is not able to duplicate itself and function again. It is resorbed along with the organelles that develop from it, while the portion of the centriole that remains within the nucleus is able to reproduce itself and, in the next cell division, to give rise to daughter axostyles, each axostyle carrying with it as it leaves the nucleus an axostyle- and flagella-producing portion of the centriole.

The Achromatic Figure

The interphase nucleus of *Oxymonas grandis,* unlike that of other species of *Oxymonas,* does not contain a karyosome. The chromatin consists of many granules which appear to be connected to or enclosed within several irregularly shaped strands of non-staining or matrix material (Fig. 1). No centrioles or centrosomes can be seen. Later, however, after the central spindle has developed, centrioles, which are circular when the central spindle is viewed from the end, may be seen at the ends of the central spindle. These centrioles are probably present in the interphase, but are difficult to differentiate from the chromatin which, at this stage, is generally distributed through the nucleus.

Previous studies of *Oxymonas* (Janicki, 1915; Zeliff, 1930; Connell, 1930) have indicated that the central spindle arises from the karyosome, but this cannot be the case in *O. grandis* since none is present, and a re-investigation of *O. dimorpha* shows that the central spindle does not arise from the karyosome as Connell (1930) thought to be the case. A number of instances were observed where the central spindle had developed to considerable size while the karyosome was still present, a situation resembling that described by Kirby (1928) in *Microrhopalodina* (=*Proboscidiella*) *kofoidi* (see Kirby's Figs. 41, 42, Pl. 24) and by Cleveland et al (1934) in *Saccinobaculus,* in which a karyosome similar to that of *O. dimorpha* is present. It might also be noted here that the central spindle of *O. dimorpha* (as noted by Cleveland et al, 1934, Fig. 446) is clearly fibrillar when sufficiently destained and is not a bar as reported by Connell (1930) in this species, by Janicki (1915) in *O. granulosa,* and by Zeliff (1930) in several species of *Oxymonas.*

The notion that the central spindle is a bar or a rod has resulted from the failure to understand how it is formed. If it were produced by the elongation of a karyosome or by the division of a centriole (or centrosome or "blepharoplast") as has been supposed to be the case, one could expect it to be bar-like or rod-like in certain instances, but it is very doubtful if the central spindle is ever formed in any cell in this manner. It is certainly formed in many, if not all, instances by fibres arising from one centriole meeting and overlapping those that arise from the other centriole. However, the central spindle in different types of cells, just as the centriole from which it arises, varies greatly, both in size and structure. The fibres composing the central spindle may be either very compact or greatly dispersed, with all gradations between these extremes; they may be few in number or numerous. And the shape of the central spindle varies from a flat or band-like structure to one that is cylindrical. The gradations from one so-called type of central spindle to another are so close—almost every conceivable step being represented when many different types of cells are considered—that there appears to be no justification for the use of different names to refer to them. For example, if one uses the term paradesmose—as was originally done by Kofoid and Swezy (1915)—to refer to rod-like extranuclear central spindles, one soon discovers every gradation from long, slender structures to large cylindrical ones, and there is no means of determining what is a paradesmose and what is a central spindle. The same is true if one attempts to use the term centrodesmose for intranuclear central spindles. Thus, the only basis left for using the terms paradesmose and centrodesmose is the fact that the former is outside the nuclear membrane and the latter is inside. But the central spindle is outside or inside the nuclear membrane not because it is a different structure nor because it functions differently, but because, in certain cells, the nuclear membrane persists during mitosis. And hence it is more accurate to state that the nuclear membrane persists during cell division than to infer, by the use of the terms paradesmose and centrodesmose, that the central spindle in these organisms differs from that of those organisms where the nuclear membrane disappears soon after mitosis begins.

The intranuclear achromatic figure of *O. grandis* is so large and clear that the manner in which it develops and the types of fibres composing it may be studied in considerable detail. The first astral rays that arise from the centrioles soon meet, join, overlap, and grow along one another to form the early central spindle which is narrow and about half as long as the diameter of the nucleus (Fig. 2). (In several nuclei, structures have been seen which are probably central spindles in a slightly earlier stage of development than the one illustrated.) As these fibres that are joined to form the central spindle increase in length, more astral rays arise from the centrioles, join, and overlap and thus the central spindle becomes longer and broader, soon extending from one edge of the nucleus to the other (Figs. 3, 4). The centrioles, however, continue to give off astral rays, some of which meet and overlap, some of which meet but do not overlap, and some of which scarcely extend to the centre of the nucleus and hence do not meet. As a result of this increase in the number of astral rays, the central spindle not only becomes broader but also more cylindrical and thus extends practically throughout the nucleus (Figs. 5–10). Owing to the fact that the fibres that overlap to form the central spindle do not arise at the same time but over a considerable period, the central spindle is frequently composed of two, three, four, or more bundles or groups of fibres (Figs. 7, 8, 10), and it is difficult in the metaphase to determine which are the fibres that first met and overlapped. However, it is sometimes possible at this stage of mitosis to differentiate clearly the first-formed portion of the central spindle from later-formed portions (Fig. 9) and, in later stages of mitosis when the nucleus is elongate, the first-formed portion can always be differentiated from later-formed portions (Figs. 12–17) since the later-formed portions pull apart earlier (due to less overlapping of the fibres). The fibres of the first-formed portion of the central spindle may be seen clearly as the developing daughter nuclei become constricted (Fig. 15), they hold together as a definite bundle of fibres as the daughter nuclei separate (Fig. 16), and persist for some time after they pull apart (Fig. 17), but disappear before the chromosomal fibres (Fig. 18).

Some of the astral rays which arise from the centrioles never meet and overlap to form the central spindle. Those which become attached to the chromosomes and function in the movement of the chromosomes toward the poles, because of their function, should be termed chromosomal fibres, and those which do not become attached to the chromosomes nor join to form the central spindle should be termed astral rays. Thus, the achromatic figure is composed of three types of fibres, central spindle fibres, chromosomal fibres, and astral rays. Of course, it must be remembered that the chromosomal fibres and the fibres composing the central spindle are merely astral rays that are performing known functions.

There are certainly no other fibres in the achromatic figure of *O. grandis,* nor in any genus of hypermastigotes previously studied (Cleveland et al, 1934), and the writer is doubtful regarding the existence of the so-called interzonal fibres. In most instances, they are the (overlapping) fibres of the central spindle which extend from pole to pole (centriole to centriole) but are sometimes more or less obscured except in the region between the two groups of daughter chromosomes. It is fortunate in this respect that the chromosomes of *O. grandis* are small and in no way interfere with an analysis of the achromatic figure. In a few instances, the daughter chromosomes of certain cells spin out fibre-like threads or strands as they separate, and until these threads pull in two, they may be mistaken sometimes for fibres. It is probable that some of the so-called interzonal fibres that have been described are these threads between the groups of daughter chromosomes, but most of them are undoubtedly nothing more than the fibres of the central spindle. And it is an easy matter to make such a mistake unless the fibres of the central spindle are many in number and fit closely together.

In the early stages of the development of the central spindle, the chromatin has more or less the same appearance as in the interphase (Figs. 1–4). But as the central spindle extends through most of the nuclear space, the chromatin begins to move toward the centre of the nucleus (Figs. 5, 6). The mechanism that carries it there is not known, although it is possible that the growth of many astral rays from each centriole pushes it to the centre; in other words, causes it to congregate between the groups of astral rays (Figs. 6–10) until some of the astral rays are converted into chromosomal fibres by becoming attached to the chromosomes.

The chromosomes, as in other species of *Oxymonas* and in *Microrhopalodina,* are many in number, small, and somewhat irregular in shape.

REFERENCES CITED

Cleveland, L. R., S. R. Hall, E. P. Sanders, and J. Collier, 1934. The Wood-feeding Roach Cryptocercus, its Protozoa, and the Symbiosis between Protozoa and Roach. *Mem. Am. Acad. Arts and Sci.,* **17**: 185.

Connell, F. H., 1930. The Morphology and Life-cycle of Oxymonas dimorpha sp. nov., from Neotermes simplicicornis (Banks). *Univ. Calif. Publ. Zool.,* **36**: 51.

Janicki, C., 1915. Untersuchungen an parasitischen Flagellaten. II. Teil: Die Gattungen Devescovina, Parajœnia, Stephanonympha, Calonympha.—Über den Parabasalapparat.—Über Kernkonstitution und Kernteilung. *Zeitschr. f. wiss. Zool.,* **112**: 573.

Kirby, H., Jr., 1926. The Intestinal Flagellates of the Termite, Cryptotermes hermsi Kirby. *Univ. Calif. Publ. Zool.,* **29**: 103.

Kirby, H., Jr., 1928. A Species of Proboscidiella from Kalotermes (Cryptotermes) dudleyi Banks, a Termite of Central America, with Remarks on the Oxymonad Flagellates. *Quart. Jour. Mic. Sci.,* **72**: 355.

Kofoid, C. A., and O. Swezy, 1915. Mitosis and Multiple Fission in Trichomonad Flagellates. *Proc. Am. Acad. Arts and Sci.,* **51**: 289.

Kofoid, C. A., 1926. On Oxymonas, a Flagellate with an Extensile and Retractile Proboscis from Kalotermes from British Guiana. *Univ. Calif. Publ. Zool.,* **28**: 285.

Zeliff, C. C., 1930. A Cytological Study of Oxymonas, a Flagellate, including Descriptions of New Species. *Am. Jour. Hyg.,* **11**: 714.

EXPLANATION OF PLATE I

Several fixatives and stains were used but all the nuclei illustrated were fixed in Schaudinn's fluid and stained either in Heidenhain's or Delafield's hæmatoxylin. Drawings were made with the aid of a camera lucida and are × 1600.

Oxymonas grandis sp. nov. from *Kalotermes* (*Neotermes*) *dalbergiæ* Kalshoven, 1930

FIG. 1. Interphase nucleus.

FIG. 2. Early central spindle extends through the central portion of the nucleus.

FIG. 3. Central spindle, longer, slightly broader, and extends all the way through the nucleus. Note centriole at left end of central spindle.

FIG. 4. Central spindle composed of more fibres and is broader. Chromatin in this and in preceding stages more or less as in interphase.

FIG. 5. More astral rays have arisen from the centrioles. Some of these will overlap to form the central spindle and some will become chromosomal fibres.

FIG. 6. Central spindle and the other fibres of the achromatic figure extend through most of the nucleus. Note circular centriole at left end of the achromatic figure (this end is tilted slightly upwards).

FIGS. 7–10. Chromatin in centre of nucleus. Achromatic figures broad at ends and extend practically throughout the nuclei. Note variations in the fibre-groups of the central spindle.

FIG. 11. Nucleus elongating, chromatin moving toward poles (centrioles).

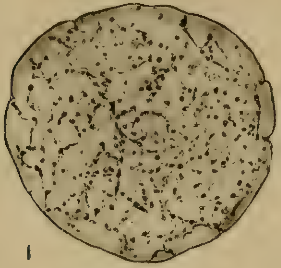
FIGS. 12–14. Nuclei more elongate, some fibres of central spindle have pulled apart, and chromosomes nearer poles.

FIG. 15. Nucleus constricted with the first-formed portion of the central spindle extending between developing daughter nuclei. Note fibres on left that extend beyond the chromosomes but do not form a part of the central spindle; these once met similar fibres from the other centriole and formed a portion of the central spindle.

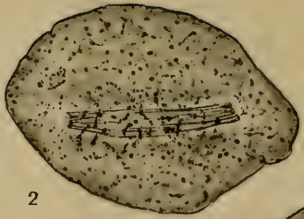
FIG. 16. Daughter nuclei have separated except for the first-formed portion of the central spindle which extends from one centriole to the other. The centriole of the daughter nucleus on the right is viewed almost from the end.

FIG. 17. Central spindle has pulled apart but has not disappeared. Daughter nuclei no longer connected. Chromosomal fibres still present.

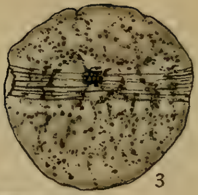
FIG. 18. Daughter nucleus shortly before cytoplasmic division. Fibres of the central spindle have disappeared but chromosomal fibres remain. Chromatin is returning to the interphase condition, although it has not reached the pole (centriole).



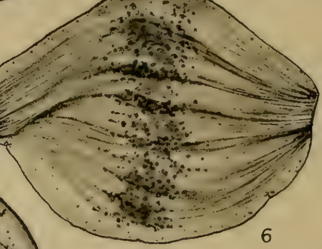
1



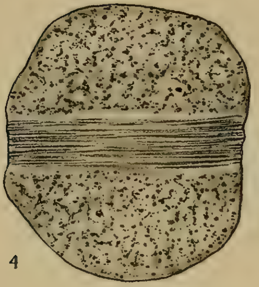
2



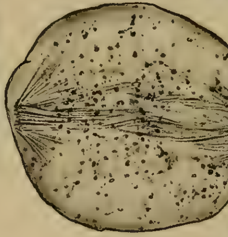
3



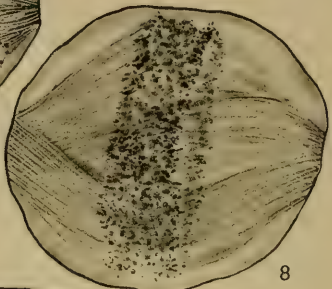
6



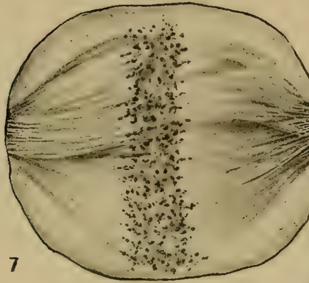
4



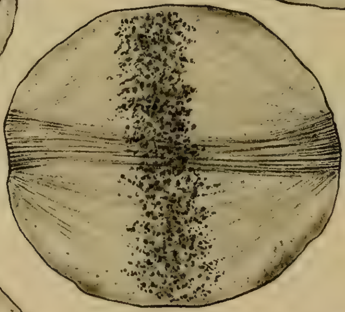
5



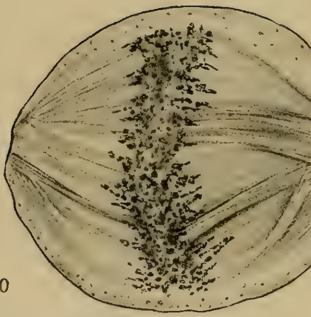
8



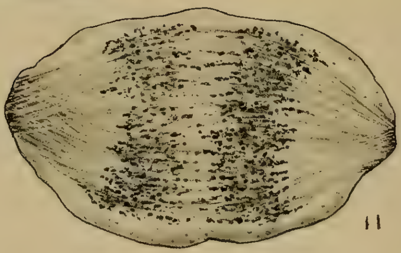
7



9

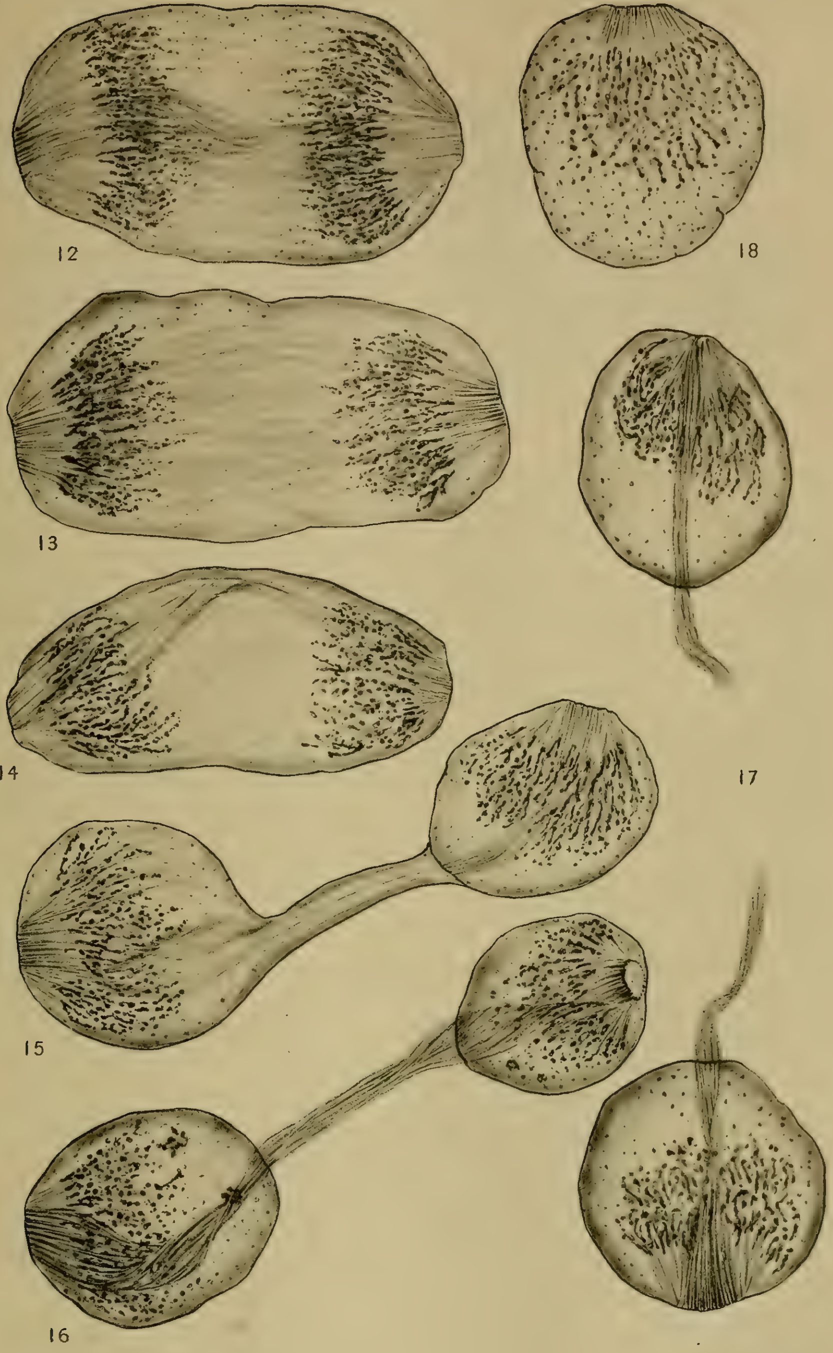


10



11

Elizabeth P. Sanders del.


12
18
13
14
17
15
16

L. R. Cleveland

ON THE HEMOLYMPH CELL COUNTS OF SOME MARINE INVERTEBRATES

J. FRANKLIN YEAGER AND OSCAR E. TAUBER

(*From the Department of Zoölogy and Entomology, Iowa State College*)

This paper contains results of investigations[1] on the hemolymphs from 26 different species of marine invertebrates. The objectives were to obtain values for the total hemolymph cell counts and for the mitotic indices of the different species.

METHODS

Total hemolymph cell counts were made with the hemocytometer technique usually employed to obtain total blood cell counts of vertebrates but with the following modifications. A special dilution micropipette, calibrated to dilute approximately 1 and 2 cu. mm. of hemolymph 150 and 75 times, respectively, was employed. The diluting fluid consisted of 20 cc. of boiled, filtered sea water plus three drops of 10 per cent acetic acid plus 3 cc. of 0.01 per cent gentian violet in boiled, filtered sea water. In the case of *Phascolosoma,* some counts were made with diluting fluid from which the acetic acid was omitted. A few counts were obtained with the usual white blood cell diluting pipette from animals subjected to sea water containing acetic acid, but as this treatment was found to be unnecessary when the micropipette was employed, it was used only a few times. Whenever signs of cell coagulation appeared either in the dilution pipette or in the counting chamber the count was not made or was discarded.

The mitotic indices of the hemolymphs were made according to the method previously applied by the authors (1933) to insect hemolymph. A drop of the acid sea water diluting fluid was submerged in clear mineral oil on a clean glass slide and a small drop of hemolymph mixed with the diluting fluid as quickly as possible after sampling; the acid fixes the cells, preventing cell coagulation, and the gentian violet stains the nuclei. When mitosis occurs, as in insect hemolymph, the pro-, meta-, ana-, and telophases can easily be recognized. The value of a mitotic index is obtained by counting 2,000 cells, taken at random, noting the number of mitotic figures encountered and expressing the result as the number of mitotic figures per thousand hemolymph cells.

---

[1] This work was aided by a grant from the Rockefeller Fluid Research Fund, administered through Iowa State College. The work was done at the Marine Biological Laboratory, Woods Hole, Mass., in the summer of 1934.

Results

The total hemolymph cell counts and the various species from which they were obtained are given in Table I. The number of total cell counts obtained is in column 2. The number of animals used is in column 3. Mean total cell counts and standard deviations are in column 4; each mean and standard deviation is derived from the corresponding group of counts recorded in column 2. In column 5 is indicated the source of the hemolymph sample used in making the count.

The number and values of mitotic indices are not given in the table since they are all the same, that is, less than 0.5, or less than 1 mitotic figure in 2,000 cells. Usually two or three determinations were made for each species, but larger numbers (15, 7, 9, 26, 5, respectively) were used for *Cancer borealis, Libinia canaliculata, Carcinus granulatus, Arbacia punctulata,* and *Phascolosoma gouldii.*

Discussion

Few total hemolymph cell counts of marine invertebrates have been found in the literature. Hardy (1892) records a count of 286 cells per cu. mm. of *Astacus* hemolymph. This value is so much less than those in Table I and the values of other invertebrate hemolymphs obtained by the authors (1932, 1933, 1934, 1935, in press) that they are inclined to think Hardy's low count was caused by loss of cells in hemolymph coagulation. In general, these counts are of the same orders of magnitude as the counts previously obtained from insects and, like the latter, they exhibit considerable variation within and between species; the intraspecies variation is indicated by the standard deviation (Table I, column 4).

The adults of *Limulus* exhibited a significantly higher mean count than did the group of eight young individuals (lengths from 13.0 to 15.0 cm.; average 14.0 cm.); the standard deviation of the difference (15,400) between the two means [2] is 2,900. This is in accordance with previous observations of the authors (1935) that total hemolymph cell counts of various observed heterometabolous insects (Orthoptera) tend to be greater in the adult than in the nymphal individuals, the average nymphal counts ranging from about 72 to about 41 per cent of the corresponding average adult counts. The *Limulus* young adult ratio of these counts is about 49 per cent.

---

[2] According to the formula $\sigma_D = \sqrt{\frac{\sigma^2 A}{N-1} + \frac{\sigma^2 N_y}{N-1}}$.

TABLE I

Hemolymph Cell Counts of Marine Invertebrates

Species (1)	Number of total hemolymph cell counts (2)	Number of animals used (3)	Mean total hemolymph cell count ± standard deviation (× 10^3) (4)	Hemolymph samples from (5)
Crustacea				
Crago vulgaris	15	13	8.3 ± 2.7	Pericardial sinus
Palaemonetes vulgaris	23	16	2.7 ± 0.8	Pericardial sinus
Peneus setiferus	27	14	8.9 ± 2.0	Pericardial sinus
Homarus americanus	68	19	18.7 ± 6.5	Antenna
Hippa talpoida	16	11	10.6 ± 4.1	Pericardial sinus
Talorchestria longicornis	12	11	5.1 ± 2.6	Pericardial sinus, antenna
Pagurus pollicaris	24	12	26.0 ± 11.5	Pericardial sinus
Cancer borealis	19	10	14.2 ± 7.2	Pericardial sinus, leg
Cancer irroratus	32	12	16.3 ± 9.6	Pericardial sinus
Libinia canaliculata	31	12	11.9 ± 5.6	Pericardial sinus
Callinectes sapidus	18	12	54.1 ± 19.6	Pericardial sinus, heart
Carcinus granulatus	13	13	31.6 ± 18.1	Pericardial sinus, heart
Ovalipes ocellatus	14	7	20.1 ± 5.9	Pericardial sinus
Uca minax	25	13	12.4 ± 4.4	Pericardial sinus
Uca pugilator	18	10	5.2 ± 1.5	Pericardial sinus
Uca pugnax	33	16	12.4 ± 6.4	Pericardial sinus
Echinodermata				
Arbacia punctulata	32	20	11.3 ± 5.6	Perioral puncture
Asterias forbesii	27	13	6.3 ± 2.4	Dorsal part of ray puncture
Mollusca				
Loligo pealii	13	9	7.0 ± 3.6	Circulatory system
Mya arenaria	8	8	20.2 ± 7.7	Heart
Venus mercenaria	5	5	9.0 ± 5.9	Heart
*Ostrea virginica**	7	6	22.4 ± 6.8	Heart
Vermes				
Amphitrite ornata	14	13	47.4 ± 20.1	Body wall puncture
Nereis virens; red fluid	5	4	29.6	Body wall puncture
Phascolosoma gouldii	33	15	78.9 ± 31.9	Body wall puncture
Arachnida				
Limulus polyphemus; adult	63	27	30.0 ± 11.4	Dorsal tail-joint puncture
Limulus polyphemus; young	8	8	14.6 ± 5.0	Dorsal tail-joint puncture

---

* The authors are indebted to Dr. P. S. Galtsoff, of the U. S. Bureau of Fisheries, for the individuals of *Ostrea* used.

In the molluscs, *Mya* and *Ostrea,* the mean total cell counts of the hemolymph obtained directly from the heart cavity exceeded similar counts (not given in Table I) of the pericardial fluid; the differences are statistically significant.

The blood of *Nereis* was difficult to sample. A colorless and a red fluid (blood) could be obtained by body wall punctures. To what extent the sample of red blood was diluted by the colorless fluid was not readily detectable but, when this was obviously the case, the count was not made. The results indicate, nevertheless, that the cell content of the red exceeds that of the colorless fluid. Counts were not made on the body fluid of *Cerebratulus* because of sampling difficulties caused by the reflex segmentation of the body when stimulated by body wall puncture.

The red cells of *Phascolosoma* tended to cytolyse rapidly in the acid diluting fluid. Omission of acetic acid from the fluid retarded somewhat but did not entirely prevent cytolysis; by working rapidly, however, it was possible to obtain counts. Operation of the cytolysis factor would tend to decrease the observed count value.

It is of interest that all determinations of mitotic index gave negative results, indicating less than 1 mitotic figure in 2,000 hemolymph cells. It would appear that the crustaceans named in Table I differ from the insects that have been investigated by the authors in that the latter yield positive results when investigated in the same way. Insects have not been shown to possess definite hemolymph cell-forming tissues while in some, at least, of the crustaceans cell-forming tissues have been described. The low mitotic indices obtained from these crustaceans are in accordance with the view that hemolymph cell production in these marine arthropods occurs chiefly in special leucopoietic tissues whereas, in the insects, it occurs chiefly by mitosis of circulating hemolymph cells.

CONCLUSIONS

1. The total hemolymph cell counts of the 26 species of marine invertebrates studied exhibit, like the total hemolymph cell counts of insects, considerable interspecies and intraspecies variation. The standard deviations given in Table I indicate the latter.

2. The counts of adult *Limulus polyphemus* tend to exceed those of young *Limulus.*

3. The mitotic indices of the species studied were found to be less than one mitotically dividing cell in 2,000 hemolymph cells.

REFERENCES

Hardy, W. B., 1892. The Blood Corpuscles of the Crustacea, together with a Suggestion as to the Origin of the Crustacean Fibrin-ferment. *Jour. Physiol.,* **13**: 165.

Tauber, Oscar E., and J. Franklin Yeager, 1934. On the Total Blood (Hemolymph) Cell Count of the Field Cricket, Gryllus assimilis pennsylvanicus Burm. *Iowa State College Jour. Sci.,* **9**: 13.

Tauber, Oscar E., and J. Franklin Yeager, 1935. On the Total Hemolymph (Blood) Cell Counts of Insects. I. Orthoptera, Odonata, Hemiptera and Homoptera. II. Neuroptera, Coleoptera, Lepidoptera and Hymenoptera. *Ann. Ent. Soc. Amer.* (In press).

Yeager, J. Franklin, and Oscar E. Tauber, 1932. Determination of Total Blood Volume in the Cockroach, P. fuliginosa, with Special Reference to Method. *Ann. Ent. Soc. Amer.,* **25**: 315.

Yeager, J. Franklin, and Oscar E. Tauber, 1933. On Counting Mitotically Dividing Cells in the Blood of the Cockroach, Periplaneta orientalis (Linn.). *Proc. Soc. Exper. Biol. and Med.,* **30**: 861.

NOTES ON DIFFERENTIAL THRESHOLD OF REACTION TO VITAMIN D DEFICIENCY IN THE HOUSE SPARROW AND THE CHICK

HERBERT FRIEDMANN[1]

(*From the Smithsonian Institution, United States National Museum*)

Inasmuch as relatively little is known of the physiology of birds other than a few domesticated forms, any such additional data bearing on wild species are worth recording. In the past two years, in the course of an experimental study of the possible antirachitic potentialities of the secretion of the uropygial gland of the house sparrow, *Passer domesticus,* and of the starling, *Sturnus vulgaris,* some facts were brought out indicating a threshold of reaction to vitamin D deficiency very different from that of the fowl.

In the course of the experiments the sparrows and the starlings were divided into groups of four birds of each kind. The two species were kept separate; in fact, the sparrows were used the year after the starlings. The work of Hou (1928 *a, b,* 1929, 1930) indicated that in young chicks the oil gland secretion was necessary for the birds to absorb ultra-violet rays and enable them to produce vitamin D, and the argument used in the work on starlings and sparrows, based on this, was as follows. If the uropygial exudate was a potential source of vitamin D, swallowed by preening the feathers on which it had been irradiated by sunlight following its application, then if young birds were experimentally induced to develop rickets, that condition could be rectified or prevented by feeding irradiated oil gland secretion.

Six groups of four birds each of both species were put in a room without direct sunlight (no ultra-violet recorded on test with a photoelectric cell); three of these groups were operated on and all trace of the oil gland removed. This left, then, three sets of two groups of four birds each of each species, one group of operates and the other of unoperates. Another set of one operated and one unoperated group was kept in an outdoor cage where the birds received the normal ultraviolet of the direct sunlight. The outdoor set and all the indoor sets were fed on a vitamin-D-free diet based on one recommended by the nutrition laboratories of the poultry husbandry department of the U. S. Department of Agriculture at Beltsville, Maryland. This diet consisted of 35 per cent fine cracked yellow corn, 25 per cent middlings, 35 per cent wheat, and a little common salt and caseine. (The caseine was not used in the experiments with the sparrows.) In addition to this, one of the indoor sets of two groups (one operates and one unoperates) was fed the equivalent of one mashed oil gland (from donor birds of the same species) per bird every other day in the case of the starlings and two per week in the case of the young sparrows. (The starlings were older than the sparrows when used in the experiments and would normally have had more of the oil gland exudate available.) Furthermore, another indoor set of two groups was fed exactly the same except that the mashed oil gland material was first irradiated with ultra-violet, the dosage used being an exposure of twenty seconds at two feet with a red purple ultra filter that transmitted nothing below the intensities found in sunlight; the source being a Cooper-Hewitt 220 volt D. C. quartz mercury arc in the laboratories of the Division of Radiation and Organisms of the Smithsonian Institution. Dr. E. D. McAlister, who very kindly assisted me in this matter of irradiating the materials, has described the intensities in the visible and ultra-violet spectrum of this lamp (1933). The idea behind these three sets of operates and unoperates was that if the uropygial exudate, when irradiated, produced vitamin D, the groups fed such irradiated substance should survive the experimental conditions without developing rickets while the other groups should exhibit rachitic symptoms. The vitamin D free diet had previously been found, at the Beltsville laboratories, to produce rickets in chicks, even when given direct sunlight, in four weeks or less. It may be said at this point that the untreated uropygial exudate reacted photochemically like a cholesterol.

---

[1] Published by permission of the Secretary of the Smithsonian Institution.

The first experiments were done with the starlings. Unfortunately the studies were begun too late in the autumn to get birds still in juvenal plumage, and so it is more than possible, even more than probable, that at least some of the individuals used were not really young birds of the year. The experiments were continued for a period of eight weeks, twice the time required to produce rickets in chicks, and not one of the thirty-two birds showed any rachitic symptoms, either locomotor, postural, or upon dissection. The bones were compared with "normal" skeletons of the same species and found to be identical.

The second series of experiments was done with definitely young house sparrows. The same procedure was followed and the experiments were continued for nine weeks. Towards the end of the seventh week a few of the birds (both operates and unoperates) appeared to be somewhat unsteady on their feet and, instead of perching on the toes alone, used the entire tarsometatarsus as well, when standing on the floor of the cage. However, autopsies, both macroscopic and microscopic, failed to reveal any trace of rickets at the end of the ninth week in these or any of the other birds. Two of those that most definitely appeared rachitic from their locomotor and postural reactions, were sent to Dr. Harry W. Titus, biological chemist of the U. S. Animal Husbandry Experiment Farm at Beltsville, Maryland. He turned them over for examination to Mr. J. C. Hammond, who had been working with him on rickets and perosis in the chicken. All Hammond's findings were negative. Silver nitrate staining showed that there had been recent calcification in the proximal head of the tibia of each bird. The bones were of normal proportions, and there was no sign of bending on the ribs. The skeletal parts of two normal birds were used as checks in all tests.

The failure of the experiments to induce rickets in any of the birds precluded, of course, any chance to test the possible nutritional importance of the uropygial exudate suggested by Hou's work, but it did bring out the fact that young house sparrows, and, much less definitely, starlings (age of birds not certain), have a markedly different threshold of reaction to vitamin D deficiency than do young chicks. Compared to the chicks, the sparrows seem relatively independent of vitamin D.

Since this work was done, a paper by Knowles, Hart, and Halpin (1935) has appeared in which the authors show that in the presence of a sufficient source of the antirachitic factor, either as direct ultraviolet irradiation or as cod liver oil in the diet, the preen gland is a dispensable organ for calcium metabolism in the chick. The situation was different in the case of the birds herein reported in that there was no possible source of the antirachitic factor in either the light or the food. It is to be expected that under normal conditions the preen gland is nutritionally dispensable as many species of wild birds do not have the gland at all, and in the case of the domestic pigeon, some individuals have one and others do not. However, those species that are so equipped may be a little less rigidly bound environmentally by the ecological presence or degree of presence of the antirachitic factor.

It is planned to extend this study to cover a variety of species, as a differential degree of necessity for vitamin D may help partially to explain specific differences in food habits and, to a lesser extent, possibly even of nutritional limiting factors in geographic distribution of certain birds.

Before concluding this paper, it must be stated that the actual work was done at the National Zoölogical Park in Washington, D. C., where, through the kind coöperation of the authorities, space, cages, and the necessary keeper's services were generously supplied me. To Mr. Malcolm Davis, keeper of the bird house, I am especially indebted for help in obtaining birds and in assistance rendered throughout the work.

LITERATURE CITED

Hou, Hsiang-Ch'uan, 1928*a*. *Am. Jour. Physiol.*, **85**: 380.
1928*b*. *Chinese Jour. Physiol.*, **2**: 345.
1929. *Chinese Jour. Physiol.*, **3**: 171.
1930. *Chinese Jour. Physiol.*, **4**: 79.
Knowles, H. R., E. B. Hart, and J. G. Halpin, 1935. *Poultry Science*, **14**: 33.
McAlister, E. D., 1933. Smithsonian Miscellaneous Collections, **87** (No. 17): 1.

THE UTILIZATION OF SOLAR RADIATION BY ALGÆ AT DIFFERENT DEPTHS IN LAKES

C. JUDAY AND H. A. SCHOMER

(*From the Limnological Laboratory of the Wisconsin Geological and Natural History Survey*)

INTRODUCTION

General investigations relating to the penetration of solar radiation into the waters of Wisconsin lakes have been in progress for a number of years. These studies have dealt with the quantity of energy that penetrates to different depths in various types of lakes and also with the spectral composition of the radiation at these depths. Four papers based on the results obtained in these investigations were published between 1929 and 1932, inclusive, and material is now in hand for a fifth report. In addition to the field studies, a laboratory investigation of the absorption at definite wave-lengths in the spectrum by settled and filtered lake waters as well as by carefully distilled water has been in progress for the past four years; the results obtained in this laboratory study are now being prepared for publication.

These studies led up to the problem of the relation of the quantity and composition of the radiation at various levels to the photosynthetic activities of the aquatic plants at these depths in different types of lakes. Experiments dealing with this problem were begun in the summer of 1932 and they were continued in 1933 and 1934. The present report is based on results obtained in three lakes in 1933.

APPARATUS AND METHODS

The quantity of radiation reaching the surface of the lakes was measured with a self-recording solarimeter and the percentage of this energy that penetrated to different depths was determined with a pyrlimnometer. Light-filters were used on the pyrlimnometer in order to ascertain the spectral composition of the radiation at different depths. The sky was fairly clear on the dates represented in these experiments.

Cultures of two species of algæ were used for the experiments, namely, *Chlorella pyrenoides* and *Coccomyxa simplex*. The algal cultures were grown out of doors in the shade. When needed for the experiments, they were centrifuged out of the culture medium and transferred to filtered water from Trout Lake. This lake water contained an average of 1.3 mg. per liter of free carbon dioxide, 38 mg. per liter of bicarbonate carbon dioxide, and 0.012 mg. per liter of nitrate N. The number of algal cells averaged 125,000, ranging from 80,000 to about 200,000 per cubic centimeter of water.

The procedure was essentially the same as that of Marshall and Orr (1928). Glass-stoppered bottles of about 150 cc. capacity were filled with the algal material and they were then suspended at different depths in the lake in wire baskets. Four bottles were used at each depth, two clear ones and two controls that were covered with black paint and a black cloth bag. The algal samples were kept at the various depths for a period of three hours. The quantity of oxygen produced during this time was determined by a modified Winkler method.

Lakes

Crystal Lake has an area of 30 ha. and a maximum depth of 21 meters. It has neither an inlet nor an outlet and the water is very soft and transparent. The disc reading at the time of the experiment was 13.5 m. The water showed no brown color whatever. The temperature ranged from 22.8° C. at the surface to 22.5° at 7 m., 17.3° at 9 m., and 15.8° at 10 m.

Trout Lake has an area of 1583 ha. and a maximum depth of 35 m. It has several inlets and an outlet. The water usually shows a slight brown color, which ranges from zero to 14 on the platinum-cobalt scale. The disc readings fall between 3.3 m. and 6.5 m., with a mean of 4.5 m. At the time of the experiments, the water had a uniform temperature of 19.5° from the surface to 10 m.

Helmet Lake has an area of 3 ha. and a maximum depth of 10 m. It is a typical bog lakelet and has no outlet. The water is highly colored; the color readings range from 168 to 268 on the platinum-cobalt scale. The transparency of the water is low; the disc readings vary from 0.8 to 1.5 m. At the time of the experiments, the temperatures were 24.0° at the surface and 21.0° at 2 m.

Oxygen Production

The results given for oxygen production in Table I show that maximum photosynthesis took place at 5 m. in Crystal Lake in the *Coccomyxa* series and at 6 m. in the *Chlorella* series. In the former experiment, 17.4 cal/cm.^{2} of the radiation delivered to the surface of the lake penetrated to 5 m. in the three-hour period, while in the *Chlorella* series 14.0 cal/cm.^{2} reached 6 m. in three hours. The radiation at 5 m. consisted of the following percentages: violet 16, blue 20,

TABLE I

Utilization of Solar Radiation by Algæ at Different Depths in Lakes

The oxygen production is stated in milligrams per million cells for a three-hour period. The energy value of the glucose is given in gram calories per square centimeter of cell surface and the solar radiation in gram calories per square centimeter for a three-hour period. Total radiation is given for the surface of the water, but only 80 per cent of this amount was used in computing results for surface samples because they were covered by a few centimeters of water.

Lake and date	Alga	Depth	Oxygen production	Glucose value of oxygen	Energy value of glucose	Solar radiation	Utilization
		meters	*mg.*				*per cent*
Crystal, Aug. 5....	Chlorella	0	0.256	0.240	0.385	134.9	0.36
		1	0.476	0.446	0.716	48.5	1.47
		4	0.508	0.476	0.765	21.2	3.60
		5	0.515	0.482	0.774	16.6	4.66
		6	0.528	0.495	0.795	14.0	5.68
		7	0.504	0.472	0.758	11.4	6.65
		10	0.359	0.336	0.540	5.9	9.15
Aug. 7..........	Coccomyxa	0	0.380	0.356	0.572	141.4	0.50
		1	0.416	0.390	0.626	50.9	1.23
		4	0.467	0.438	0.703	22.2	3.16
		5	0.501	0.470	0.755	17.4	4.34
		6	0.477	0.447	0.718	14.7	4.88
		7	0.475	0.445	0.715	12.0	5.96
		10	0.343	0.321	0.515	6.2	8.30
Trout, Aug. 23.....	Chlorella	0	0.053	0.050	0.083	190.4	0.05
		1	0.216	0.202	0.324	55.2	0.58
		2	0.224	0.210	0.337	34.1	1.00
		3	0.223	0.209	0.335	22.6	1.48
		5	0.210	0.196	0.315	9.7	3.25
		7	0.135	0.126	0.202	4.7	4.30
		10	0.051	0.048	0.077	1.9	4.05
Aug. 28.........	Coccomyxa	0	0.063	0.059	0.095	122.1	0.09
		1	0.145	0.136	0.218	35.4	0.61
		2	0.134	0.125	0.200	22.0	0.91
		3	0.130	0.121	0.194	14.5	1.34
		4	0.117	0.110	0.176	9.4	1.87
		5	0.100	0.094	0.150	6.2	2.42
		10	0.050	0.047	0.075	1.2	6.25
Helmet, Aug. 11....	Chlorella	0.00	0.166	0.155	0.249	191.1	0.16
		0.25	0.254	0.238	0.382	24.8	1.54
		0.50	0.235	0.220	0.353	7.6	4.64
		0.75	0.162	0.152	0.244	4.8	5.10
		1.00	0.133	0.125	0.200	2.1	9.52
		2.00	0.050	0.046	0.074	1.6	4.62
Aug. 14.........	Coccomyxa	0.00	0.299	0.280	0.450	236.3	0.23
		0.25	0.413	0.387	0.621	30.7	2.02
		0.50	0.372	0.348	0.559	9.4	5.94
		0.75	0.238	0.223	0.358	5.9	6.06
		1.00	0.196	0.184	0.295	2.6	11.34
		2.00	0.076	0.071	0.114	1.8	6.33

green 27, yellow 23, orange 10, and red 4. Thus 50 per cent of the energy fell in the green and yellow bands of the spectrum, which are usually regarded as the most favorable region for photosynthesis.

In Trout Lake, maximum yields of oxygen were obtained at depths of 1 m. and 2 m. in the two series as indicated in Table I, but they were much smaller than those found in Crystal Lake. The amount of solar radiation, on the other hand, was much larger at 1 m. and 2 m. in Trout Lake than at 5 m. and 6 m. in Crystal Lake, so that the maximum production of oxygen was not proportional to the amount of radiation at these depths in the two lakes. In Trout Lake, however, the amount of radiation at 2 m., the point of maximum production on August 23, was only a little smaller than that at 1 m. in the *Coccomyxa* series of August 28. At 1 m. in Trout Lake the radiation had the following percentage composition: violet 9, blue 17, green 18, yellow 21, orange 20, and red 15. In this instance only 39 per cent of the energy fell in the green and yellow bands of the spectrum.

In the highly colored water of Helmet Lake, the algæ produced the maximum quantity of oxygen at a depth of one-fourth meter in both series. The *Coccomyxa* yield on August 14 was larger than that of *Chlorella* on August 11; this was accounted for, in part at least, by the larger amount of radiation on the former date. The solar radiation was absorbed very rapidly by the water of this lakelet; it was reduced to 0.8 per cent of the amount delivered to the surface at a depth of 2 m. At 1 meter 78 per cent of the energy was in the red band of the spectrum, 18 per cent in the orange, 3.5 per cent in the yellow and a small amount in the green.

UTILIZATION OF SOLAR RADIATION

The quantity of oxygen produced at the different depths and the amount of solar radiation that penetrated to these levels give a basis for the estimation of the percentage of energy utilized by the algæ during the three-hour periods of the various experiments. The oxygen values given in Table I include that which was liberated in the manufacture of carbohydrates as well as that which was used by the algal cells in respiration as shown by the control samples. The amount of oxygen consumed in respiration was not as large in the cool lower water as in the warm upper stratum; a decrease of 5–6° in temperature produced a decline of about 15 per cent in the oxygen consumption. The total oxygen production has been converted into terms of glucose with a combustion value of 3760 calories per gram.

The surface area of the cells has been used in the utilization computations because the chloroplasts of these two forms of algæ are hollow, cup-shaped structures which occupy the greater part of the cell. The yield of oxygen is stated in fractions of a milligram per million cells with a surface area of 2.34 $cm.^2$.

The radiation fell principally upon the upper surface of the algal cells, but a certain amount reached them laterally by reflection. A few observations, however, indicated that the amount of lateral radiation was relatively small. Radiation from below was cut off by the black bottles of the control samples. Thus the solar energy affected about three-quarters of the surface of the cells. The entire surface has been used in the computations because the cultures were kept in motion by the action of the waves on the buoy from which they were suspended. This shaking of the algal suspensions prevented any permanent orientation of the cells with respect to the source of the radiation.

The last column of Table I shows the percentage of energy utilized by the algæ at the various depths during the three-hour periods. In Crystal Lake, the percentage of utilization gradually increased from a minimum of 0.3 per cent at the surface to a maximum of 9.1 per cent at 10 m. in the *Chlorella* series of August 5, and from 0.5 per cent at the surface to 8.3 per cent in the *Coccomyxa* series of August 7. In both cases the maximum percentage was found at 10 m., where the solar energy was only 4.3 per cent of the amount delivered to the surface of the lake. On the other hand, the smallest percentage of utilization was found in the surface samples which were covered by only a few centimeters of water so that they received approximately 80 per cent of the radiation that reached the surface of the lake.

The water of Trout Lake was less transparent than that of Crystal Lake, so that a smaller amount of radiation penetrated to depths of 5 m. or more. While the oxygen yield was smaller in Trout than in Crystal, a similar percentile increase in utilization with increasing depth was noted in Trout Lake. The percentage rose from 0.05 per cent at the surface to a maximum of 4.3 per cent at 7 m. in the *Chlorella* series of August 23 and from 0.09 per cent at the surface to 6.2 per cent at 10 m. in the *Coccomyxa* series on August 28.

Likewise the percentage of utilization increased with increasing depth in Helmet Lake, but the maximum percentage was found at a depth of only 1 m. in both series represented in Table I. In both experiments the maximum percentages were larger than those observed in Crystal and Trout lakes; they are considerably larger than both of the Trout Lake series, but the *Chlorella* maximum of Crystal Lake is almost as large as that of Helmet Lake. The *Chlorella* sample at 1 m. utilized 11.3 per cent of the radiation which reached that depth on August 14.

In Crystal Lake, the utilization reached a maximum percentage where the total radiation for the three-hour period amounted to about 6.0 cal/cm.^{2}; in Trout Lake the maxima were correlated with 4.7 and 1.2 cal/cm.^{2}, respectively, and in Helmet Lake with 2.1 and 2.6 cal/cm.^{2}. These results seem to show, therefore, that the highest percentage of utilization of solar radiation by the phytoplankton of a lake falls somewhere in the stratum that receives from 1.2 to 6.0 cal/cm.^{2} in a three-hour period during the middle of the day. The increase in the percentage of utilization with increasing depth, and the consequent decrease in the amount of radiation, are in accordance with the results obtained by Warburg and Negelein (1922); they found that a larger percentage of energy was utilized by *Chlorella* in a weak than in a strong illumination. They also observed a higher percentage of utilization in the red than in the blue part of the spectrum. Clarke and Oster (1934) give oxygen results for two experiments with diatoms, but their measurements of the radiation cover only the blue and red parts of the spectrum so that the values can not be compared directly with these from Wisconsin lakes.

With respect to the spectral composition of the radiation at the depths where maximum percentages of utilization were obtained, 4 per cent of the energy at 10 m. in Crystal Lake was in the violet part of the spectrum, 26 per cent in the blue, 25 per cent green, 32 per cent yellow, 11 per cent orange, and 2 per cent red. At 10 m. in Trout Lake, 3 per cent was violet, 32 per cent blue, 30 per cent green, 27 per cent yellow, 7 per cent orange, and 1 per cent red. In Helmet Lake, on the other hand, 78 per cent of the energy at 1 m. was red and 18 per cent orange, with small amounts of yellow and green. Thus 57 per cent of the energy at 10 m. in both Crystal and Trout lakes fell in the green and yellow bands, but only 4 per cent was present in these two bands at 1 m. in Helmet Lake. This indicates that the maximum percentage of utilization is not correlated directly with the amount of energy in the green and yellow color bands. This agrees with the results obtained by Miss Stanbury (1931) for *Nitzschia closterium;* she found that the growth of this diatom was roughly proportional to the energy received, irrespective of its wave-length in the visible spectrum.

SUMMARY

1. The depths of maximum oxygen production and of maximum percentage of utilization of solar radiation were determined for *Chlorella pyrenoides* and *Coccomyxa simplex* in three lakes.

2. The maximum yield of oxygen was obtained at a depth of 5–6

m. in the most transparent lake and at 0.25 m. in the one with highly colored water.

3. The maximum percentage of utilization of solar radiation was found at a depth of 10 m. in the more transparent waters and at 1 m. in the highly colored water.

4. The highest utilization was 11.3 per cent in the colored water and 4.3 to 9.1 per cent in the other two lake waters.

LITERATURE CITED

Birge, E. A., and C. Juday, 1929. Transmission of Solar Radiation by the Waters of Inland Lakes. *Trans. Wis. Acad. Sci.,* **24**: 509. Second report. 1930. *Ibid.,* **25**: 285. Third report. 1931. *Ibid.,* **26**: 383. Fourth report. 1932. *Ibid.,* **27**: 523.

Clarke, G. L., and R. H. Oster, 1934. The Penetration of the Blue and Red Components of Daylight into Atlantic Coastal Waters and its Relation to Phytoplankton Metabolism. *Biol. Bull.,* **67**: 59.

James, H. R., 1935. A Laboratory Study of the Absorption of Light in Lake Waters. Ms.

Marshall, S. M., and A. P. Orr, 1928. The Photosynthesis of Diatom Cultures in the Sea. *Jour. Mar. Biol. Ass.,* **15**: 321.

Schomer, H. A., and C. Juday, 1935. Photosynthesis of Algæ at Different Depths in Some Lakes of Northeastern Wisconsin. I. Observations of 1933. *Trans. Wis. Acad. Sci.,* **29**: In press.

Stanbury, F. A., 1931. The Effect of Light of Different Intensities, Reduced Selectively and Non-selectively, upon the Rate of Growth of Nitzschia closterium. *Jour. Mar. Biol. Ass.,* **17**: 633.

Warburg, O., and E. Negelein, 1922. Über den Energieumsatz bei der Kohlensäureassimilation. *Zeitschr. phys. Chem.,* **102**: 235.

REGENERATION IN THYONE BRIAREUS LESUEUR FOLLOWING INDUCED AUTOTOMY

FRANK R. KILLE

(From Hull Laboratory, University of Chicago, and the Marine Biological Laboratory, Woods Hole, Mass.)

INTRODUCTION

It has long been known that many holothurians have the ability to cast out a large portion of their viscera and to regenerate all the lost organs (Dalyell, 1840, 1851; Semper, 1868; Noll, 1881; Scott, 1914; Crozier, 1915; Bertolini, 1930, 1932). Although the fact of such regeneration is well known, there have been but few attempts to analyze the manner in which autotomized parts are reconstituted (Scott, 1914; Bertolini, 1930, 1932), and there is even less data on the mode of regeneration following experimental operations (Monticelli, 1896; Torelle, 1909). During the summers of 1931 and 1932 I had an opportunity at Woods Hole[1] to make such investigations upon *Thyone briareus,* a holothurian common to that region.

Our knowledge of the way in which the lost parts are reconstituted in *Thyone briareus* is confined to the accounts of Torelle (1909) and Scott (1914). Scott (1914) described the regeneration within whole animals following self-evisceration, beginning with a stage 9 days after evisceration. Previously, Torelle (1909) described briefly the regeneration within posterior halves of bisected *Thyone* but described earlier stages than did Scott. We have then no account of the early stages of regeneration within whole animals and only a brief note on these stages as found in the regeneration within posterior halves. Furthermore, disagreement on the part of these two authors as to certain details in the later stages of regeneration raised the question as to whether regeneration within the whole animal is comparable to that within a posterior half. Thus the problem was twofold: (1) to ascertain the manner in which *Thyone* regenerates autotomized parts with especial reference to the early stages of such reconstitution and (2) to compare the regeneration occurring within a whole animal with that which takes place within a posterior half. With these two general problems in mind, a study was made of the regeneration within whole animals following induced evisceration and also of the regeneration within anterior and posterior halves of *Thyone,* resulting from a cut made before or after evisceration.

---

[1] This work was begun at Woods Hole in the summer of 1931 through the aid of a Collecting Net Scholarship.

I am deeply indebted to Dr. B. H. Willier for helpful advice and criticism given throughout the course of the investigation.

Auto-evisceration in Holothurians

There is much variation among holothurians in respect to the ease with which autotomy is induced. In *Stichopus regalis* the process is apparently a normal one taking place naturally every year toward the end of the summer (Bertolini, 1932*a*). In the laboratory this species will eviscerate readily when irritated by some means such as increased temperature of the water. It often exhibits autotomy when it is caught (Bertolini, 1930*b*). In speaking of *Holothuria captiva* Ludwig, now *H. parvula* Selenka (Deichmann, 1930, p. 70), Crozier (1914, p. 199) states that "even when kept in well ærated sea water aquaria protected from the light, holothurians will eviscerate after about four days."

In contrast to such forms, *Thyone briareus* has been kept at least a month in running water aquaria without exhibiting autotomy. Furthermore, among some 400 medium-sized specimens which were examined during the course of this investigation I found none that was in a natural state of regeneration. Since *Thyone* are greatly reduced in volume after evisceration, regenerating animals may have been eliminated through this selection for size. Only a very large specimen would escape it. The time of year may be another factor. In the instances of *Stichopus regalis,* Bertolini (1932*a*) found individuals in stages of regeneration during some seasons while at other seasons none was in this condition. She reported that of 50 animals collected in the spring none was in a state of regeneration, yet out of 119 collected during September and October, 110 were regenerating and 7 had recently eviscerated. It is possible then that regenerating *Thyone* might be collected in other seasons even though none was found in mid-summer. Pearse (1909) has stated that "it seems improbable that autotomy is an important factor in the daily life of the members of either genus" (i.e., *Thyone* or *Leptosynapta*).

Methods for Inducing Evisceration in Thyone

In order to obtain a complete series of animals showing progressive stages in the process of regeneration, it was necessary to find a stimulus effective enough to initiate autotomy but which would not lead to the death of the animal. Pearse (1909) injected various chemicals into the body-cavity of *Thyone briareus.* He found that many substances which produce " intense contractions of the muscles of the body-wall, did not bring about ejection of the visceral organs " and " under the best of conditions it appears in only 35 per cent of the possible cases." The highest percentage of evisceration was obtained by the use of strychnine, seven out of twenty showing autotomy and living for at least a day. Scott (1914), working with this same species, induced autotomy by " allowing *Thyone* to stand in stagnant water until the water became foul." This was followed by " treatment with running water containing much oxygen. Alternating these processes produced as high as 65 per cent of self-mutilated individuals." These animals lived and regenerated the lost parts. It has been shown (Kille, 1931) that if *Thyone* were placed in dilute ammonia water autotomy almost invariably followed. If the animals were returned to sea water following this treatment, 96 per cent of them lived and regenerated the lost parts. In this same note (1931) it was also stated that electrical stimulation applied to the muscles of the body-wall will produce autotomy. Electrical stimulation proved to be as satisfactory as the use of ammonia water except that the ammonia method was the more rapid. Recently an electrical stimulus has been used successfully to induce evisceration in several species of Holothuria (Bertolini, 1932*b*) which, like *Thyone,* seldom if ever undergo autotomy.

Evisceration Induced by Ammonia Water

When *Thyone* is disturbed the anterior region of the body-wall is invaginated by the contraction of the five retractor muscles extending from the radial plates of the lantern to the longitudinal muscles of the body-wall (Figs. 1 and 2). The introvert (the invaginated anterior

---

EXPLANATION OF PLATE I

Photographs 2, 8, 9, and 10 from preserved material; all others from motion picture film of living *Thyone* by J. R. Brewster. All figures 4/5 natural size except Figs. 2 and 7, which are 3/5.

FIG. 1. *Thyone briareus* with introvert retracted. Anterior end to the right. *p,* position of the posterior border of the introvert.

FIG. 2. Dissection to show position of introvert. *a,* anterior border of the introvert.

FIGS. 3, 4, 5, AND 6. Successive stages (intervals of 2 seconds) in the eversion and distention of the introvert during autotomy.

FIG. 7. Bursting of the introvert (at *b*). Small portion of the intestine visible.

FIG. 8. Autotomized anterior end of *Thyone* and viscera.

FIG. 9. Autotomized viscera partially disentangled in order to show lantern (*l*), polian vesicle (*p. v.*), stomach (*s*), tentacles (*t*), and intestine (*i*).

FIG. 10. An eviscerated *Thyone* opened along the right dorsal interambulacrum. Water lungs have been removed. Arrows indicate the mesentery. *g,* gonad tubules; *c,* cloaca.

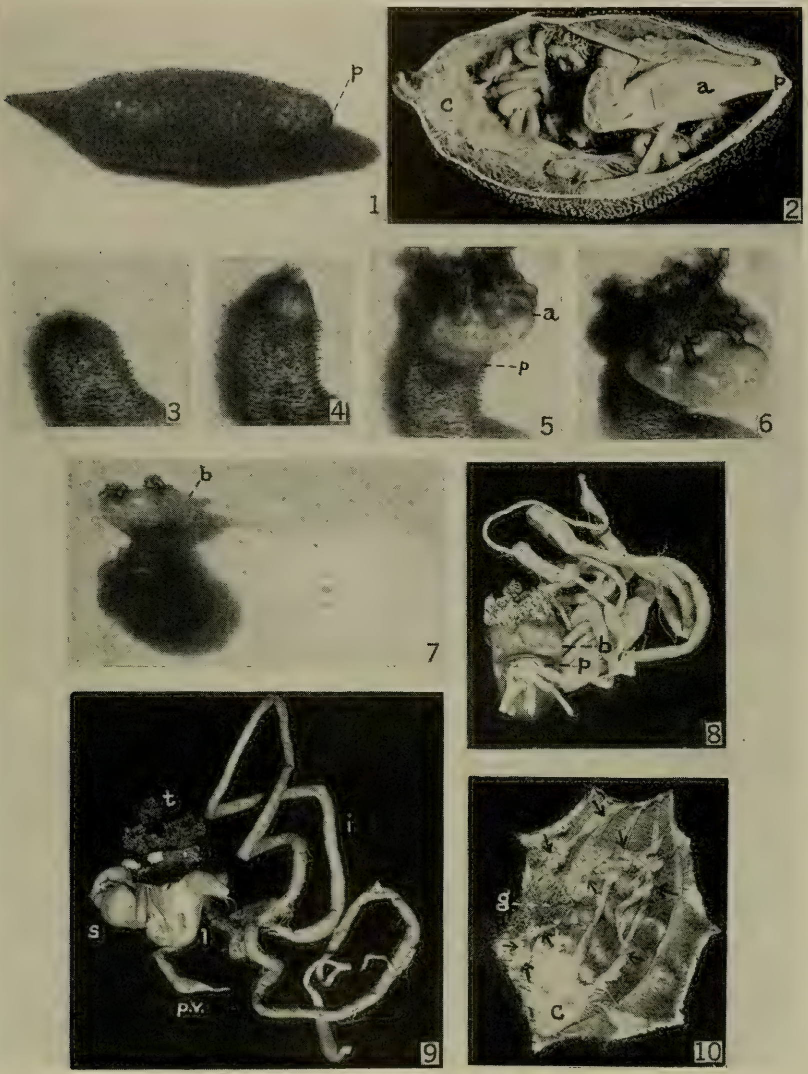

p
1
c
a
p
2
3
4
a
p
5
6
b
7
b
p
8
t
i
s
l
p.v.
9
g
c
10

PLATE I

region of the body-wall, Fig. 2, *a-p*) possesses no tube feet and lacks the strong circular muscles which characterize the rest of the body-wall. The animal in this condition was submerged in weak ammonia water. Ammonium hydroxide was used in the proportion of one part 7 N. ammonia to 800 parts of sea water. Within about 15 seconds all body muscles are thrown into strong contraction as a result of which the body-wall is much wrinkled. The animal becomes reduced to a minimum size and assumes a spherical shape. In this condition the antero-posterior axis is about one-third the length of that of a fully extended specimen.

This period of extreme contraction continues for some 15 seconds. At the close of this period the invaginated anterior end becomes rapidly everted (Figs. 3, 4, and 5) and then greatly distended (Fig. 6) as the intestine, stomach, lantern, and body fluid are forced anteriorly into this region by the further contraction of the strong circular and longitudinal muscles of the body-wall. It is impossible to say at just what moment the retractor muscles lose their attachment to the longitudinal muscles of the body-wall and the gut is separated from its mesentery and cloaca. In order to throw some light upon these points three specimens were killed in the act of autotomy by the injection of 20 cc. Bouin's fluid into the body-cavity. In all specimens the introvert was evaginated and partly distended when fixed. Dissection showed that in one specimen all retractor muscles were detached but the posterior section of the intestine was not. It still retained its connection to the cloaca and its mesentery. On the other hand, in another specimen one retractor (the ventral) was still attached but the entire gut free from mesentery and cloaca was lodged in the expanded introvert. The third specimen, unlike the first, had only two detached retractor muscles but resembled the first in that the posterior end of the gut was still joined to the cloaca and its mesentery. It would seem from this evidence that there is no absolute sequence between the autotomy of the retractors and the autotomy of the gut, both events being completed some time during the last few seconds of the period of intense muscular contraction preceding evagination of the introvert or during the early stages in the autotomy of the body-wall following rupture. Furthermore, it is apparent that the actual separation of all the retractors from the body-wall does not necessarily precede the evagination of the introvert.

The distended introvert usually ruptures (Fig. 7, *b*). The body-fluid gushes forth carrying with it variable amounts of the viscera. The circular muscles of the body-wall contract so that the body-cavity is closed posterior to the rupture (region *p*, Fig. 2). The ruptured anterior end containing the viscera is sloughed off within several hours, but can be very easily pulled away from the rest of the body immediately after its distention. When artificially completed in this way the whole process of autotomy usually takes place within a period of 35 seconds.

The place of rupture varies. If it occurs in the anterior (Fig. 5, *a*) or middle region of the introvert (Fig. 7, *b*) then the rupture of the body-wall has a position independent of its autotomy. Autotomy of the body-wall always occurs at the transition zone between the thin-walled introvert and the muscular body-wall (Fig. 5, *p*). Figure 8 shows the autotomized parts from a specimen in which the rupture of the body-wall (at *b*) and the region of autotomy (at *p*) are independent of each other. However, the distended introvert may burst either in the region of autotomy (*p*) or so close to it that autotomy of the body-wall is completed by the extension of the original rupture.

Evisceration Induced by Means of Electrical Stimulus

This method satisfactorily induced autotomy but was more time-consuming. The animals had to be stimulated individually whereas in the case of ammonia water a group could be treated at one time. Furthermore, the electrical stimulus had to be given for a somewhat longer period of time before autotomy occurred. The procedure consisted in applying a tetanizing faradic current to various parts of the body-wall. The application was made by means of two platinum wire electrodes which were about 2 mm. apart. The anterior, posterior, and middle regions of the body were touched successively but without any regard to a specific order. In the mid-body region the applications were made on or near all five radii which mark the position of the longitudinal muscles. As soon as the muscles contracted in the region stimulated the electrodes were moved to a new position, thus keeping all muscles of the body in a state of partial or complete contraction. Following a period of intense contraction self-evisceration occurred as has already been described for the ammonia method.

THE RESULTS OF AUTOTOMY IN THYONE BRIAREUS

The autotomized parts are much entangled and are partially covered by the introvert (Fig. 8). When these parts are disengaged (Fig. 9) one sees that they include the entire length of the intestine[2] with its hæmal vessels; the stomach; the introvert; the lantern with its associated structures. Under this latter category are included water-vascular ring; Polian vesicle or vesicles; stone canal; madreporite; tentacles; œsophagus with its investment of calcareous plates; nerve ring; and the retractor muscles of the lantern. I have never seen the gonads ejected but this has been reported for an unidentified species of *Thyone* (Noll, 1881).

---

[2] For the present purposes the terminology of Coe (1912) and others will be retained. The so-called intestine thus includes at least three regions which are morphologically distinct in the living animal.

Examination of the autotomized parts also shows that they are usually eviscerated in the order of their body position and not according to their anatomical sequence. Lantern and stomach are eviscerated first, followed by the much-coiled intestine of the two regions *B* and *F–H* (Fig. 11) which are interconnected by hæmal vessels, then intestine in region *C–E,* and last of all that from the region *H–J.*

After autotomy of the viscera the animal consists of the body-wall minus the introvert; a cloaca with the attached respiratory trees; the two gonadal tufts, one on either side of the dorsal mesentery; and nearly all of the mesentery. This condition following self-evisceration is seen in Fig. 10. The specimen has been cut open longitudinally along the right dorsal interambulacrum. The respiratory trees have been removed at the point of their attachment to the cloaca in order that the distribution of the mesentery might be more easily followed. The situation is more clearly seen in Fig. 11. The respiratory trees and the entire left side of the body-wall have been removed from an eviscerated specimen. For convenience in description ten arbitrary points have been designated along the free edge of the mesentery by the letters *A* through *J.* For purposes of orientation three other structural features are marked: namely, the region of the gonad attachment (*g*), the cloaca (*c*), and the ventral longitudinal body muscle (*v*). The diagram represents the animal in its typical shape. Actually, however, the animal assumes a more spherical shape following evisceration. There is also

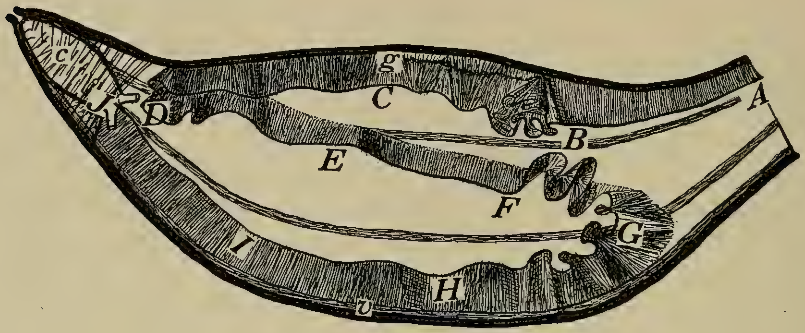


FIG. 11.

a decrease in volume due to loss of much of the cœlomic fluid. As a result the mesenteries hang in abundant folds and the straight-line distance from the anterior end to the cloaca is reduced one-half or more.

Since the regeneration of the intestine involves the free edge of the mesentery, it is advisable to follow its distribution in detail. For the purpose of description the mesentery in *Thyone* may be divided into three sections. The first (dorsal) section of the mesentery attached to the dorsal interambulacrum extends from the anterior end of the body nearly to the cloaca (Fig. 11, *A–D*). The anterior third of this section (region *A–B,* Fig. 11) supported the lantern and the short, muscular stomach. The remainder (*B–D,* Fig. 11) suspended the anterior part of the intestine. These original relationships can be established when examining an eviscerated *Thyone* by referring to the hæmal vessel in the dorsal mesentery which extends from the gonads to the hæmal vessels of the alimentary canal. In the process of evisceration this strand of hæmal tissue is severed near its attachment to the viscera. The severed end at the free edge of the mesentery (posterior to *B,* Fig. 11) marks approximately the original position of the junction between stomach and intestine. The second (lateral) section of the mesentery which supported the middle third of the gut turns to the left, passes ventrally and anteriorly across the two left longitudinal muscle bands to a point far anterior (Fig. 11, *D–G*). At this point (Fig. 11, *G*) the mesentery makes a turn to the right and as the third section (ventral) runs posteriorly to the cloaca in the left ventral interambulacrum along the ventral longitudinal muscle (Fig. 11, *G–J*). This section supported approximately the posterior third of the intestine. An occasional specimen is found in which the third section of the mesentery is attached in the right ventral interambulacrum instead of the left. Such a distribution was described by Coe (1912).

Preparation of Materials

Using the two methods described above, 150 *Thyone* were eviscerated and immediately placed in running sea water. Five failed to survive the treatment. Of those which lived, groups of six were killed and fixed in Bouin's or Zenker's fluid at time intervals of 1 day up to an 11-day regeneration, and then at every second day to a regeneration of 32 days. In the process of fixation two methods were used. In the one first adopted the animal was cut open longitudinally through the right dorsal interambulacrum so as not to disturb the mesenteric attachments to the body-wall. The specimen was sewed or pinned out flat upon a piece of cork so that the entire body cavity was exposed.

The whole preparation was then turned upside down upon the fixing solution. In the older stages of regeneration a few cuts were made through the intestine to aid in fixation. By this method the regenerated viscera were fixed entire and in those specimens which had regenerated for 12 days or more all relations were preserved.

For the earlier stages of regeneration this method could not be followed because it fragmented and distorted the fragile rudiments and frequently pulled the mesenteries from their attachments. The procedure adopted for such specimens was as follows. A small amount (4–6 cc.) of Bouin's fluid was injected directly into the body-cavity. The needle of the syringe was inserted through the right body-wall so as to avoid the mesenteries. The animal was then immersed in Bouin's fluid. The tendency for the animal to contract was offset by the slightly increased cœlemic pressure due to injection. After a short interval a small window was cut through the body-wall on the right side. This was carefully enlarged until through it the mesenteries and primordia of the new alimentary canal could be seen. When their position was determined nearly all the body-wall could be removed from the right side without danger of their mutilation. The animal was then placed in fresh Bouin's. This method not only preserved the parts in their proper relations but also in their typical position. It proved so satisfactory that it was eventually adopted for fixation of all stages of regeneration.

In the main, the data as compiled in the present paper were obtained by means of an examination of the fixed specimens under a binocular dissecting microscope. Histological analysis of the regenerating tissues is not offered at this time but certain preliminary findings of this nature will be included.

Regeneration Within an Eviscerated Thyone

Early Stages in the Regeneration of the Stomach-intestine (*2 to 10 days*)

With the aid of a dissecting microscope (× 23) one can see a fine thread of opaque tissue along the edge of the mesentery in specimens which have regenerated only 2 days. This opaque thread appears distinct with a diameter slightly greater than the thickness of the mesentery. The margin of the mesentery is quite even or regular as compared with the more or less ragged, irregular free edge resulting from the autotomy of the gut. Four and 5 days after evisceration the early morphological indication of the regenerating alimentary tract can be seen with the naked eye. When examined under the dissecting microscope it appears in the form of a continuous rod-like thickening of the free edge of the mesentery from *A* to *J*. The diameter of the rod is fairly constant but with some local variations here and there (Fig. 12, *I*). Evidently the entire free edge of the mesentery early exhibits a physiological activity which within 4 days finds morphological expression in the form of this rod-like thickening of the mesenteric edge.

While the edge of the mesentery early exhibits stages of regeneration, it also undergoes a general reduction in its total length. It will be recalled that the antero-posterior axis of the animal following evisceration is greatly reduced. The mesentery is thrown into many folds which are particularly noticeable in regions *D, G,* and just posterior to *B* (Fig. 11) where the mesentery formerly supported small loops of the intestine. These folds of the mesentery are gathered into numerous small tucks at the free margin where they are hemmed by the fine, straight thread or rod of regenerating tissue. In this way the length of the free edge of the mesentery becomes considerably reduced. The tucks are present in regenerations of 4 and 5 days but become much more prominent as regeneration advances.

Correlated with this general reduction in marginal distance from *B* to *J* there is a noticeable increase in the distance from the free edge of the mesentery to the line of attachment at the body-wall. While this growth in width is somewhat general it is pronounced in regions throughout *C–D–E* and *F–G–H*. One can easily see by referring to Fig. 11 that if growth occurs in these regions, and if at the same time the free edge is gathered into numerous tucks, the more or less hairpin turns of the free edge at *D* and *G* would be replaced by a gentle *S*-shaped curvature (Fig. 13, *B–D′–G′–I*). This *S*-shaped curve of regenerating mesenteric margin occupies a body level formerly occupied by only the middle third of each section of the mesentery. In this way the marginal distance from *B* to *J* has been further reduced. Some specimens which had regenerated for a period of 6 and 7 days showed this condition well advanced (Fig. 12, II) and it was characteristic of all specimens examined 8 and 9 days after evisceration (Fig. 12, III). In some instances (Fig. 12, IV) the rudiment of the stomach-intestine is almost a straight rod connecting the healed anterior end and the cloaca.

For detailed analysis of the marginal primordium eight specimens were selected, two for each regeneration period of 4, 6, 8 and 10 days. After examination under the binocular microscope the rudiments were removed and studied in greater detail by means of serial sections. Figure 12 shows four of these rudiments drawn to scale as seen from the right side. The mesenteries have been omitted but the ten letters assigned to arbitrary points of the free edge of the mesentery in Fig. 11 are placed along the mesenteric side of the rudiments to indicate the approximate position of these same points. No detail of the lantern is attempted. It will be seen that the rod-like primordium of the alimentary canal 4 days after autotomy has a diameter varying between the extremes of .05 mm. and .23 mm. and averaging about .11 mm.

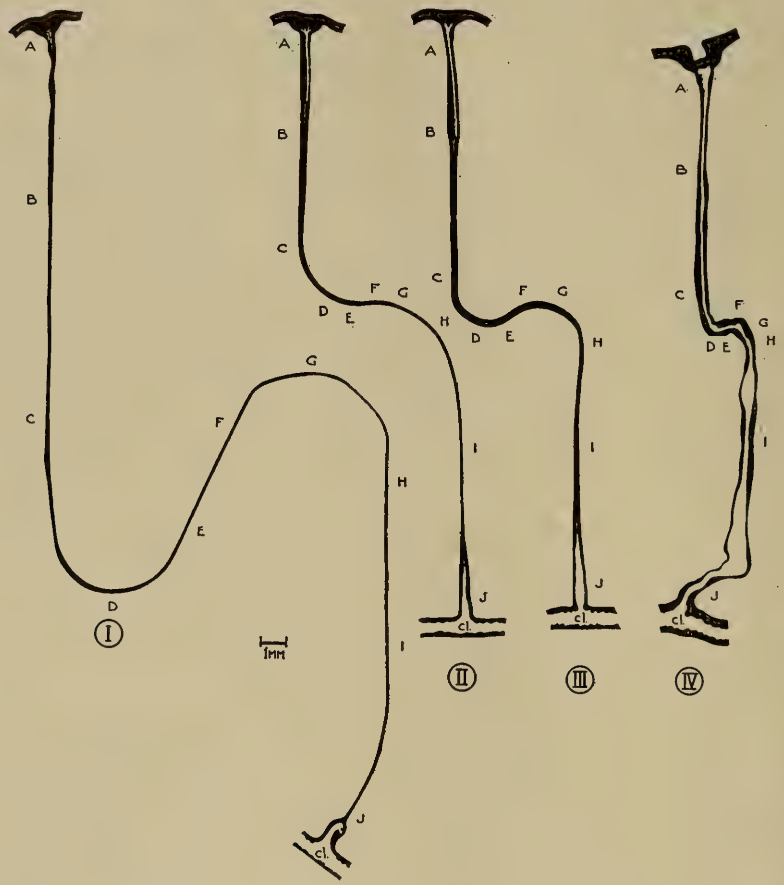


FIG. 12.

(Fig. 12, *I*). The anterior section of the rudiment is larger than that adjacent to the cloaca. There is no morphological indication here that this thickening at the edge of the mesentery was progressively established as an anterior growth from the cloaca as has been suggested for *Thyone* (Torelle, 1909). On the contrary, it would indicate a nearly equivalent activity throughout the length of the mesentery granting slight advantage to the anterior third. When the rudiment from specimen *A* was sectioned it was found to be a solid rod of connective tissue covered by a much-thickened mesothelium. At the posterior end it was continuous with the wall of the cloaca at a point where the original intestine was torn away. Here the lumen of the cloaca ended blindly. At the healed anterior end, however, certain masses of cells appear to be penetrating this solid rudiment in a posterior direction. They extend as a number of finger-like projections from irregular masses of cells located around and between the healed anterior extremities of the ambulacral structures. The large size of the nuclei and the granular cytoplasm of these invading cells stand in sharp contrast to the surrounding connective tissue cells. They seem to be more intimately associated with the water vascular canals than any other structures. Within these cords of cells which thus penetrate the extreme anterior end of the stomach-intestine anlage to a depth of .7 mm., small intercellular spaces occur.

Serial sections of Specimen II reveal clearly that the primitive lumen for the new alimentary canal arises in two centers. One center is located at the extreme anterior end within the lantern rudiment and extends posteriorly. The forerunners of this lumen are the small intercellular spaces noted in Specimen I. This section of lumen measures .8 mm. at its greatest diameter and extends longitudinally for 2.7 mm. Cross sections show it to be roughly circular in outline throughout most of its extent with a simple epithelium composed of cells identical in appearance with those composing the solid cord-like masses described above for Specimen I. Anteriorly the lumen occupies a central position within the rudiment but as it passes posteriorly it moves toward the anti-mesenteric margin so as to take an eccentric position. The second center for the origin of a lumen is found at the extreme posterior end of the rudiment extending anteriorly from the cloaca for a distance of 2 mm. and is lined with an epithelium which is continuous with that of the cloaca. Approximately 17 per cent of the total rudiment possesses a lumen in this specimen.

In Specimen III the invasion of the solid rudiment is more advanced. The position and size of the two sections of lumen are indicated in the figure. Twenty-eight per cent of the total length now contains a lumen. In another specimen which had regenerated for 8 days this value was 45 per cent.

In Specimen IV there is one continuous lumen from the anterior end to the cloaca. A plate of connective tissue still closes off the lumen of the canal at the extreme anterior end. This does not persist much longer, for as early as 16 days after evisceration a mass of dark gray ingested material was observed in both the stomach and intestine of two specimens. All specimens which had regenerated 20 days gave this macroscopic evidence of ingestion. One individual of this period was seen feeding in the aquarium.

Later Stages in the Regeneration of the Stomach-intestine (12 to 32 days)

About 12 to 14 days after evisceration the regenerating stomach-intestine shows an enlargement for a short distance posterior to the lantern anlagen. This is the forerunner of the muscular stomach. At 10 days the transition from the stomach region to the intestine is a gradual one. In one specimen at 12 days, and in all specimens of a longer period of regeneration, there is an abrupt transition from an anterior enlargement to the smaller diameter characteristic of the intestine. In these instances the stomach anlage is sharply marked off from the intestine. Eighteen days after evisceration, the stomach in most specimens is not only a distinct region of the regenerating tract but it also shows externally an extremely muscular posterior section typical of the normal.

Macroscopically the later changes in the intestine are mainly those resulting from increased length due to growth in certain regions. These intestinal regions are comparable to those in the normal animal where the intestine is thrown into numerous small loops. This is noted to some extent just posterior to *B* (Fig. 11) and also in the region of *G*. It is especially marked in the region of *D*. Just as in the normal, the regenerated intestine in these regions is early thrown into small loops so that the regularity of the *S*-curved gut is lost. It will be recalled that in the early stages of regeneration the free edge of the mesentery is gathered into numerous small tucks in these three regions, greatly reducing the length of the mesentery edge. Now, as the regenerating intestine at the margin of the mesentery grows in length, these tucks or folds become smoothed out and tend to disappear. The increased length of the intestine is first noticeable in the region D' (Fig. 13) which is approximately at the gonad level in a specimen of 14 days regeneration. As this loop of the intestine lengthens it extends farther and farther posterior from the gonad and is eventually thrown into a number of minor loops. In one 18-day regeneration the intestine extended posterior from the gonad and showed two distinct, small loops (D'', Fig. 13).

Concurrently with this growth in length of the intestine from D' to D'' the intestinal hæmal plexus is established within the first major loop.

At 14 days one can identify within the first large elbow of the *S*-curved gut, a crescent-shaped membrane which in texture resembles the mesentery (Fig. 13, *X–Y–Z*). One slim horn of the crescent (*X–Y*) extends far anterior along the anti-mesenteric surface of the first main section of the intestine while the other tapering horn is attached to the anti-mesenteric surface of the second major section of the gut (*Y–Z*). The posterior convex margin of the crescent is attached to the anti-mesenteric surface of the gut loop (D'). The concave anterior margin of the crescent is free (*Y*). Both the distribution and the nature of the membrane soon change. In two specimens regenerated for 18 days the curved gut in the region D'' (Fig. 13) is no longer attached to the posterior, convex margin of the crescent. The gut in this region has extended its length posteriorly from the gonad and apparently the crescent membrane has not kept pace. Both the anterior and the posterior borders of the membrane are now free (*Y* and *R*). Furthermore, the tissues of the membrane show condensations in the form of a plexus of strands. In the two specimens being discussed much of the membrane intervening between these strands has disappeared, leaving a net-like plexus. As a result of these two changes the former crescent-shaped membrane now extends across the body-cavity as a bridge-like plexus connecting the first two major sections of the intestine. This is the forerunner of the intestinal hæmal plexus.

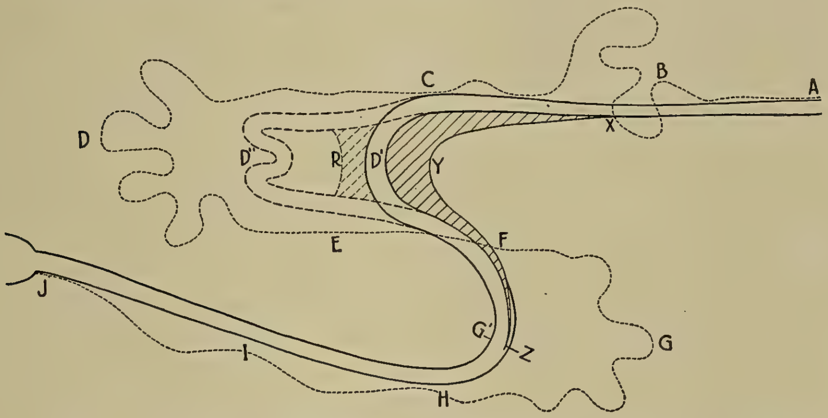


Fig. 13. $A–D–G–J$, the free edge of the mesentery as in Fig. 11; $A–D'–G'–J$, the relative position of a regenerated intestine 14 days after autotomy; $A–D''–G'–J$, th same 20 days after autotomy.

Stages in regeneration from 22 to 32 days show no marked changes except the continued elongation of the intestine and a general increase in size of all rudiments. None of the specimens had a regeneration period to exceed 32 days. Scott (1914, p. 293) states that "Thyone A, killed at 41 days, was practically a normal, both in behavior and appearance, except for the fact that the regenerated organs had not yet reached full size."

Regeneration of the Lantern and Associated Structures

Immediately after autotomy of the viscera, the circular muscles of the body-wall in the region of *p* (Fig. 2) contract strongly so as to close the body-cavity anteriorly. Within 24 hours most specimens have successfully healed the wound by means of a small circular, plate-like connective tissue mass.

The events occurring at the healed anterior end are best seen when the animal has been dissected so as to gain an internal, posterior view of the tissues in that region. From this aspect one can see the broken ends of all five longitudinal muscles which peripherally border upon the central connective tissue platelet. The anterior extremities of these muscles mark the points at which the ambulacral structures were torn across at the time of autotomy of the body-wall. The central platelet is probably derived from the neighboring connective tissue of the body-wall. The free edge of the dorsal mesentery terminates anteriorly in a fusion with the center of this tissue mass. From these three sources, the anlagen of the lantern structures arise.

As early as 5 days after autotomy one notes that each of the five radial water-vascular canals has budded a pair of small lateral vesicles at the level where the longitudinal muscle terminates, and in addition has extended its own length beyond that termination. As each canal grows anteriorly, all five canals converge centripetally towards a central point on the posterior surface of the connective tissue platelet while the paired lateral vesicles enlarge, establishing a ring of ten vesicles around its margin. These ten vesicles are the rudiments of the tentacles, which therefore arise as buds from the radial canals.

As the growing tips of the radial canals converge toward a common center, each branches dichotomously. The branches unite to form a "pentagonal canal" as has been described and figured by Scott (1914, p. 292, fig. 4).

> "The anterior ends of the radial canals fork dichotomously, and these branches anastomose to form the canal which later assumes a circular shape around the esophagus."

Usually by the time such anastomoses occur the free edge of the mesentery continuous with the center of the connective tissue platelet has developed a definite thickening so that the canal surrounds a definite enlargement of the mesentery margin from which will develop a part of the œsophagus. The appearance of the lantern anlagen at this stage reminds one of a dished-in wagon wheel on the end of an axle—the axle in this case being the solid rod-like thickening of the free edge of the mesentery, the hub being prospective ring canal, the spokes represented by the five radial canals of the lantern, while the ten tentacular vesicles indicate the circumference of the rim. When the radial canals first branch, their forked ends lie in nearly the same plane as do the tentacular vesicles, a plane which would be at right angles to the antero-posterior axis of the animal. The lantern rudiment in these early stages is therefore quite flattened, with the tentacular vesicles and ring canal forming two concentric circles about the free edge of the mesentery at *A* (Fig. 11). As regeneration advances, it is quite clear that the pentagonal canal surrounding the regenerating alimentary tract at the mesentery edge is moving more and more posterior from the plane passing through the tentacular vesicles. By the time the pentagonal canal has assumed the circular shape characteristic of the water-vascular ring, the lantern rudiment is no longer flattened but has acquired a definite antero-posterior length. The proportion is still not that of the normal lantern. In the typical lantern the maximum diameter is roughly one-half the length, while the ratio of these measurements of a regenerating lantern possessing a well-formed ring canal and tentacular vesicles may be exactly the reverse. The apparent movement of the ring canal posteriorly is associated seemingly with a relative greater growth in the region of the lantern between the ring canal and the tentacular vesicles, a region crossed by the five radial canals. At all events, the lantern rudiment eventually changes from a somewhat flattened tissue mass in which structures lie nearly in a plane at right angles to the body axis, to the form of an inverted truncated cone with its base attached anteriorly to the body-wall and its body proper projecting posteriorly into the body-cavity.

The tentacular vesicles mark the anterior level of the lantern anlagen, while the ring canal lies at the posterior limit just as in the normal adult lantern. As stated above, the tentacular vesicles arise near the termination of the longitudinal muscles. It results then that the five longitudinal muscles of the body-wall are attached peripherally at five points about the anterior end of the regenerating lantern. As early as 8 days after evisceration one can see that the longitudinal muscles have started to split off a slender inner strand from the main muscle mass. These strands of muscle become the retractor muscles of the lantern as described by Scott (1914). The splitting apparently begins a short distance from the lantern, and continues towards the lantern and posteriorly as regeneration advances. The anterior limit is soon reached at the termination of the longitudinal muscle. The splitting continues posteriorly along each longitudinal muscle thereby constantly increasing the length of the retractor muscles. All four specimens which have regenerated 14 days possessed retractor muscles about 4 mm. long. They were present in all later stages. In no specimen was there ever observed any signs of retractor muscle regeneration in the regions where the original retractors severed their attachments to the longitudinal muscles.

Twelve to 14 days after evisceration ten centers of calcification appeared anteriorly in the lantern establishing a minute calcareous ring. The ten centers mark the positions of the five radial and the five interradial plates of the completely formed lantern.

Polian vesicles originate as interradial evaginations of the water vascular ring. They can be identified in all specimens which have regenerated 12 days or more. The number of vesicles varies from one to three, as is true of the adult. The stone canal apparently originates as a dorsal diverticulum of the ring canal and is seen projecting into the dorsal mesentery. In a specimen which had regenerated 14 days the lengths of two Polian vesicles are .2 mm. and 1.2 mm. and the diameters are .12 mm. and .64 mm. respectively. The stone canal has a diameter of .12 mm. and a length of 1 mm. The distal blind end of the stone canal is slightly enlarged. In a specimen which had regenerated 16 days, that part of the dorsal mesentery between the stone canal and the regenerating lantern has disappeared so that the stone canal now borders the edge of the dorsal mesentery which hangs free in the midlantern region. Specimens at 28 days have a well developed calcareous madreporite plate of typical form.

By 14 days then, almost all rudiments of the conspicuous lantern structures are established. Most of these appear as a miniature of the typical structure. There are of course certain differences in shape so that even if the small size were ignored, the lantern at this stage could still be identified as being in the process of regeneration. The first specimen was seen feeding by means of its small, unpigmented, regenerated tentacles 20 days after autotomy.

Regeneration Within Posterior Portions of Thyone

The earliest paper on regeneration in *Thyone briareus* was that of Torelle (1909). This investigator cut off and discarded the anterior part of the body. She found that the posterior part which contained

" the intestine, the reproductive organs, and the respiratory trees " could regenerate new anterior structures. Obviously such an operation removes a bit more of the body-wall than the amount lost through the process of autotomy. Furthermore, the resulting posterior part might possess a portion of the intestine although Torelle found that " in many individuals the intestine was autotomously severed near the cloaca immediately after the operation." In spite of the reduction in the amount of body-wall and the presence of a portion of the original intestine, one would expect that such posterior halves would regenerate in much the same manner as does an eviscerated, whole animal. Yet in this brief note quite a different picture is given. Because of these differences it was thought desirable to again study regeneration within posterior sections of *Thyone briareus.*

Two series of animals were prepared. In Series *A,* the anterior ends of twenty-four eviscerated animals were amputated by a transverse cut at the level of the gonad (Fig. 11, *g*). Since there is no external indication of the attachment of the gonad, there was some variation in the cut. In all cases the lantern and associated structures were removed and sometimes the gonads. One remnant of the mesentery left within the posterior portion has a *C*-shaped distribution (Fig. 11, *C–D–E*) while the other remnant is the posterior portion of the ventral mesentery (*H–I–J*). The same operation was performed upon twenty animals of Series *B* but these were uneviscerated *Thyone.* The major difference between the two groups was that in series *B* the posterior halves retained more or less of the original intestine. This series was a repetition of Torelle's procedure. Immediately following the operation the two parts of the animal were placed in a finger bowl of running sea water. The anterior parts of the animals composing Series *B* died within 96 hours after the operation but nine of those from Series *A* lived until they were killed for examination. In the case of four anterior halves this was a period of 36 days. The posterior halves from each series possessed an equivalent viability. Approximately 80 per cent survived the operation in each instance.

Examination of the posterior portions of eviscerated *Thyone* (Series *A*) revealed a type of regeneration entirely in agreement with that observed within a whole animal. The complete rod-like rudiment of the stomach-intestine is established by an early activity which always involves the entire free edge of the mesenteric remnants. If the cut was made considerably posterior to the level of the gonad, then the rudiment along the remnant of the ventral mesentery (*H–I–J*) was easily found but that formed in the region of *D* was inconspicuous as a small portion at the extreme anterior end of the animal, i.e., immediately continuous with the regenerating lantern. This latter contribution is not readily recognized in the early stages of regeneration unless the animal is opened with extreme care. The slightest strain on the section of the mesentery *C–D–E* will pull it away from the rudiment which is held in place by its attachment to the cloaca, to the healed anterior end, and to the remainder of the mesentery. As a result the rudiment of the alimentary canal appears to be a contribution entirely from the remnant of the ventral mesentery, *H–I–J*. Later stages are less fragile. These show clearly that all mesentery posterior to the level of the cut plays a part in the establishment of the alimentary canal. This is more easily seen when the cut is at the level of the gonad attachment or just anterior to this point. The portions of the dorsal and left lateral mesenteries which were posterior to the cut form a rod-like thickening at their free edge. When viewed from the right side this thickening resembles a *C*. The rudiment along the edge of the remnant of the ventral mesentery appears as a straight rod, the anterior end of which is fused to the ventral arm of the *C*. Thus the typical *S*-shaped distribution of the original intestine is regained although imperfectly at first. Instead of a letter *S*, the rudiment resembles a reversed numeral 2.

These regenerating posterior portions further resemble regenerating whole animals in respect to the origin of the intestinal lumen. As was the case in whole animals, this lumen was found within the otherwise solid thickening of the mesenteric margin in two widely separated regions. One section is adjacent to and continuous with the cloaca, while the other is within the anterior portion of the rudiment, extending posterior from the lantern primordium. Thus the primitive lumen of the intestine is not established as a progressive development from the cloaca although the posterior portion may so arise. The lantern regeneration is usually delayed some 10 days as compared with its reconstitution within a whole animal. It was commonly observed that these animals transversely bisected in the region of the gonad experienced some difficulty in healing shut the cut end. This delay may account for the longer period required by a posterior half to regenerate a lantern. The retractor muscles are formed as in a regenerating whole animal.

Observations on the twenty specimens composing Series *B* agree with those on Series *A* when allowance is made for the parts of the original intestine which may remain. In four specimens such remainders consisted of only a short length attached to the cloaca. In the others a variable amount of the original gut was retained but never all of that which originally occupied the posterior half of the animal. Wherever a piece of the original intestine remained attached to the mesentery, this piece was incorporated into the rudiment of the new alimentary tract. Thus sections of old gut may alternate with newly regenerated links.[3] The mesenteric edge wherever free eventually bore a rod-like thickening of regenerating tissue. In some instances, sections of the original intestine became incompletely detached from the mesentery so that one end was still held by the mesentery while the other end projected into the body-cavity. That part which remained attached to the mesentery was incorporated as a link in the newly formed intestine. Usually the part extending into the body-cavity ended blindly. The new digestive system may in this way receive contributions from uneviscerated parts of the old intestine, although the origin of a lumen within the newly regenerated sections is not dependent upon such parts.

As noted above, nine anterior halves from Series *B* lived until they were killed for examination. In one of these at 11 days after the operation a small rod-like rudiment was identified along the edge of the mesentery. Such evidence from these isolated anterior halves shows that the formation of the rudiment is entirely independent of the cloaca or any posterior remnant of the original alimentary canal. The anterior halves which had regenerated for 36 days showed a well developed lantern and associated structures, stomach, and intestine but there was no indication of a cloaca.

Discussion

In respect to the regeneration of the lantern proper, my observations support those of Torrelle (1909) and Scott (1914), namely, that it regenerates from the severed anterior ends of the five sets of ambulacral structures. However, these investigators give two very different accounts of the manner in which the lantern retractor muscles arise. When examining regeneration within posterior portions of *Thyone,* Torelle (1909, p. 20) observed that:

> "if short pieces of the old lantern muscles have been left attached to the longitudinal muscles at the time of operation, the ends of these begin to proliferate new tissue about the time that the new lantern forms. . . . If the animal had been divided just posterior to the lantern-muscles, so that these had been entirely removed, a proliferation of the tissues of the longitudinal muscles takes place, in the form of a bud, at a point homologous with the position of the lantern-muscles in the normal animal. In either case the new muscles grow forward in three or more separate strand-like parts, which unite into one just before union with the lantern is effected."

---

[3] This has also been observed in three whole animals in which small remnants of the original intestine had been retained.

In contrast to this account, Scott (1914, p. 291) observed that within a whole *Thyone* following autotomy " the anterior end of each of the longitudinal muscles had split off a very slender branch to form a new retractor muscle (see fig. 3)." On this point my findings in the cases of both posterior halves and whole animals support those of Scott.

In regard to the formation of a new intestine, I find that regenerative activity begins along the entire free edge of the original mesentery. A rod-like thickening of the mesenteric margin is well developed 4 days after autotomy, forming a continuous rudiment extending from the healed anterior end to the cloaca. This analysis is quite different from that presented by Torelle (1909). Studying regeneration within posterior halves, she observed that " the new intestine always forms as a bud from one side of the old intestine, at a point near the cloaca " and it " grows forward as a solid rod of cells from one to two millimeters in diameter " until it " has become attached to the anterior closed end of the body-wall." Then " it elongates and turns on itself forming the loops characteristic of the normal animal." In contrast to this emphasis upon the importance of remnants of the original alimentary tract in the establishment of the primary rudiment, I would stress the potentialities of the mesentery throughout its full extent in whole animals as well as in posterior halves. There is evidence that a contribution is made by the cloaca in the formation of the primitive epithelium of the posterior portion of the intestine but none to indicate that this solid rod of cells at the margin of the mesentery forms as an anterior growth from a cloacal bud. If it does, it must complete its anterior progression within 2 days after autotomy. Such rudiments would be microscopic while the " solid rod of cells " described by Torelle was " one to two millimeters in diameter." The fact that a solid rudiment forms at the edge of the mesentery in an anterior half of *Thyone* which was cut off immediately following autotomy, demonstrates that neither cloaca nor any other remnant of the original alimentary canal need be present for its origin.

The question of the origin of the intestinal epithelium is, of course, another matter. It remains to be seen whether or not the intestinal epithelium which arises in an anterior half is equivalent to that within a regenerating whole *Thyone*. The evidence indicates that in whole *Thyone* an intestinal epithelium and lumen is progressively established within the posterior levels of the solid rudiment by an anterior invasion of cells from the cloacal epithelium. We have seen, however, that this contributes but a portion of the intestinal epithelium, for anteriorly the primary, solid rudiment is invaded by cells which arise within the regenerating lantern. While the exact source of these cells has not as yet been determined, the possibility that they come from any remnant of the original alimentary canal is excluded. Nothing but body-wall and mesentery is present in this region after autotomy.

The statement (Torelle, 1909, p. 19) "as soon as attachment to the anterior closed end of the body-wall is effected" the new intestine "elongates and turns on itself forming the loops characteristic of the normal animal" raises a puzzling question as to the changes that must take place in the mesentery. It is possible that this conclusion was reached as a result of observation of regeneration along the straight edge of the ventral mesentery (*H* to *J*, Fig. 11) and a failure to note the part played by the other mesenteric remnant (*C* to *E*). When the rôle of the mesentery is recognized, the eventual distribution of the new intestine is easily understood. Obviously, if the regeneration of the new intestine involves the entire edge of the mesentery which formerly supported the original intestine, then the new alimentary tract will "form the loops characteristic of the normal animal." In respect to the primary rudiment, most of the difficulties of interpretation are, therefore, eliminated when one thinks in terms of the potencies of the mesentery rather than contributions from a remnant of the original alimentary tract, i.e., the cloaca.

The part played by the mesentery in the establishment of a solid rudiment connecting the healed anterior end with the cloaca is a consistent one whether in whole animals or parts of animals. No situations have been observed where one could be absolutely sure that the rudiment ever departed from the mesentery to grow out through the cœlom independently. Every instance where this seemed to be a possibility proved otherwise. Upon careful examination it was seen that most of these misleading conditions were artificially produced as a result of too great a tension on the mesentery during fixation or examination. In early stages of regeneration the slightest tug upon the mesentery may pull it free from the rudiment at its margin. This is particularly true of the material while it is still alive. Scott (1914) noted that the new alimentery canal in a regeneration of 9 days is located along the mesenteric margin but Torelle (1909) does not mention the mesentery in her account of the earlier stages. Bertolini (1932*b*), in a passage cited below, has described for the genus *Holothuria* a situation in which the rudiment growing posteriorly from the anterior end leaves the mesenteric margin, cuts across the cœlomic cavity to another section of the mesentery, and continues on its way. It thus entirely avoids a certain region of the original mesentery but rejoins the mesentery at another point. To date I have observed no instance of this in *Thyone briareus*. In an attempt to test the possibility, sections of the mesentery were removed but in all these cases the mesentery grew rapidly from minute fragments which remained close to the body-wall and a typical regeneration followed. In carrying out this particular operation, animals which had eviscerated were turned inside out so that the mesenteries hung free in the water. Sections of the mesentery which were removed were trimmed as close to the body-wall as possible with the aid of fine shears. The animals were then turned rightside out.

The formation of the lantern at the healed anterior end of the body is probably independent of any regenerative processes occurring in various levels of the mesentery posterior to it. Torelle (1909, p. 19) pointed out that during regeneration within posterior halves the lantern never began to form "before the intestine had become united to the body wall," i.e., to the body-wall of the anterior end. The comment is made that "the attachment of the intestine appears to be a stimulus which results in . . . a proliferation of cells" to form "the beginning of a new lantern." This statement loses all significance when one finds that there is no forward growing intestine and therefore no moment when it becomes "united to the body wall." The rudiment adjacent to the body-wall in this region is as well developed in all stages as that along the mesentery at any other level. The apparent dependence of the lantern upon the regenerated intestinal rudiment within a posterior half of *Thyone* can be explained entirely upon the basis of a time relationship existing between the closure of the anterior end of the body and the activity of regeneration along the edge of the ventral mesentery. As has been stated above, the posterior portion of a *Thyone* resulting from a bisection at the level of the gonad requires considerably more time to successfully heal the wound and close the body anteriorly than does a whole animal following autotomy. Regeneration of the lantern is delayed some 10 days. On the other hand, this transverse cut does not proportionally decrease the activity of the edge of the ventral mesentery. The result is that in most specimens there will be established a sizable primordium of the intestine from the level of the cut to the cloaca before healing and regenerative processes will have established the lantern anlagen.

The recent investigations of Bertolini make it possible to compare the regeneration of the new intestine in *Thyone briareus* with like processes in the genera *Stichopus* and *Holothuria*. When *Stichopus regalis* undergoes autotomy, the cloaca tears. The intestine along with the respiratory trees and reti mirabili are emitted through the anus. In the *Holothuria* the left respiratory tree is retained. In both genera the œsophagus and cloaca remain in the body. The digestive system of *Stichopus regalis* is not regenerated from these remnants of the original tract, however, but forms in the mesentery at the expense of cells which are not distinguishable from other cells of the mesenchyme (Bertolini, 1930*b*). Specimens which had just undergone autotomy could not be kept alive in the laboratory for more than a day or so. Fortunately, Bertolini was able to secure from the sea four animals in various stages of regeneration. In the first stage an irregular cavity was found in the mesentery at its free margin. No internal epithelium lined this cavity and its aspect was practically the same at all levels. The second stage described is that of a small transparent tube seen macroscopically to run the full length of the mesentery. A definite internal epithelium was seen in a third animal which represents a still later stage in regeneration. The author points out that in all three specimens the development is equivalent at all levels of the mesentery. From this the deduction is made that the epithelium of the new intestine did not have its origin from that which remained in the œsophagus and cloaca.

In contrast to this, in *Holothuria tubulosa* (Bertolini, 1932*b*), a thin, transparent tube grows posterior from the œsophagus, avoids all the mesentery which formerly held the loop of the stomach [4] and passes along the length of the mesentery which previously held the intestine. At the same time another thin tube with a blind end starts from the cloaca and runs forward along the mesentery which formerly held the last portion of the original intestine. After about 60 days the two tubes meet and unite to form a single tube extending from the œsophagus to the cloaca. At 4 months the digestive apparatus appears to be normal again. Successive, late stages were not obtained so that the steps in the regeneration of the stomach are unknown. We do not know, therefore, whether the mesentery which finally supports the new stomach is the old mesentery or a new one.

In these two genera we see quite different methods in regeneration. *Thyone briareus* presents a third variation, for it has something in common with each, yet it presents a distinct mode of regeneration of its own. In respect to the establishment of the primary solid rudiment extending from the healed anterior end to the cloaca, *Thyone* somewhat resembles *Stichopus*. That is, the entire edge of the mesentery becomes active without much variation at different levels. This is not true for the origin of the intestinal lumen and epithelium. In respect to these, *Thyone* resembles the genus *Holothuria* in that they arise in two centers which are located at the extremities of the animal. The closer homology is found in the origin of the epithelium from the cloaca. In respect to the origin of the epithelium at the anterior end the similarity is one of position only, for in *Thyone* there is no anterior remnant of the original alimentary tract to contribute the invading cells which establish the anterior section of the intestinal epithelium. At the present stage of investigation I am unable to state the source of these invading cells. In the region where they are first seen we have the healed severed ends of all the ambulacral structures which include muscle, nerve, water-vascular and the so-called hæmal tissue as well as the mesentery.

---

[4] Nomenclature of Enriques (1902) was used.

It is of interest to recall that the embryonic origin of the water-vascular system is a vesicle which at an early stage separated from the primitive archenteron. Both intestinal epithelium and the lining of the water-vascular system are therefore closely related ontogenetically. However, one should build no argument on this platform, for the fact that structures which embryonically arise from one germ layer may regenerate from a different one has long ceased to occasion surprise. From the numerous examples the situation in the nemerteans may be called to mind. Coe and others have reported that if an anterior piece of a nemertean be cut off at such a level that it contains none of the digestive system, this piece will reconstitute a complete worm. The new midgut is formed from the mesenchyme and phagocytic cells of a posterior blastema (Coe, 1934).

SUMMARY

1. Autotomy of the stomach, intestine and lantern with its associated structures was uniformly induced in *Thyone briareus* by chemical or electrical stimulation. Ninety-six per cent of the animals lived and regenerated the lost parts.

2. Although the body-wall may burst in various regions of the introvert, actual autotomy of the body-wall always occurs at the junction between the introvert and the muscular body-wall.

3. The earliest macroscopic indication of the regenerating digestive system takes the form of a continuous, rod-like thickening of the free margin of the mesentery connecting the healed anterior end and the cloaca.

4. A lumen arises within the extremities of the primary, solid rudiment. The intestinal epithelium lining the posterior section of lumen is continuous with the cloacal epithelium while at the anterior end the intestinal epithelium arises from certain masses of cells identified within the lantern rudiment.

5. The solid rudiment is progressively invaded from these two centers until 10 days after autotomy the two sections of lumen have joined to form a continuous lumen for the new alimentary canal.

6. The lantern and associated structures originate from contributions

of the body-wall and more especially from the anterior extremities of the ambulacral structures.

7. A posterior half of an eviscerated or uneviscerated *Thyone* will regenerate all missing structures in a manner entirely comparable to that occurring within an entire animal except that regeneration of the lantern is delayed.

8. Remnants of the original intestine remaining in posterior halves cut from uneviscerated animals may become incorporated as links in the newly-formed digestive system. This has also been observed within incompletely eviscerated whole animals.

9. Anterior halves of eviscerated *Thyone* killed at 37 days after the operation possessed a well developed lantern with its associated structures, stomach, and intestine but no cloaca.

10. A comparison is made between the methods of regeneration found in the genera *Stichopus, Holothuria,* and *Thyone.*

LITERATURE CITED

Bertolini, F., 1930a. Rigenerazione dell' apparato digerente nelle Oloturie. *Rend. R. Acc. Lincei* (Cl. Sc. fis. mat. and nat.) S. 6, **11**: 600.

Bertolini, F., 1930b. Rigenerazione dell' apparato digerente nelle Stichopus regalis. *Pub. Siaz. Zool. Napoli,* **10**: 439.

Bertolini, F., 1931. Rigenerazione dell' intestino nelle Oloturie. XI. Congr. *Intern. Zoologia, Padova 1930, Atti,* **2**: 421.

Bertolini, F., 1932a. L'autotomia dell' apparato digerente e la sua rigenerazione nelle Oloturie, come fenomeno spontaneo e normale. *Rend. R. Acc. Lincei* (Cl. Sc. fis. mat. and nat.) S. 6, **15**: 893.

Bertolini, F., 1932b. Rigenerazione dell' apparato digerente nelle Holothuria. *Publ. Staz. Zool. Napoli,* **12**: 432.

Coe, W. R., 1912. Echinoderms of Connecticut. *Conn. Geol. and Nat. Hist. Survey,* Bull. 19.

Coe, W. R., 1934. Regeneration in Nemerteans. IV. *Jour. Exper. Zoöl.,* **67**: 283.

Crozier, W. J., 1914. A Note on the Physiology of the Cuvierian Organs of Holothuria captiva Ludw. *Am. Jour. Physiol.,* **36**: 196.

Crozier, W. J., 1915. The Sensory Reactions of Holothuria surinamensis Ludwig. *Zool. Jahrb., Abt. f. allg. Zool. u. Physiol.,* **35**: 233.

Dalyell, Sir J. G., 1840. Ueber die Wiedererzeugung von verlorengegangenen Organen, welche die Functionen von Kopf und Eingenweiden besorgen, bei Holothirien und Amphitriten. *Froriep's Neue Notizen aus dem Gebiete der Natur und Heilkunde,* **16**: 1.

Dalyell, Sir J. G., 1851. The Powers of the Creator Displayed in the Creation. Vol. 1, London.

Deichman, Elizabeth, 1930. The Holothurians of the Western Part of the Atlantic Ocean. *Bull. Mus. Comp. Zoöl., Harvard College,* **71**: No. 3.

Enriques, P., 1902. Digestione, circolazione e assorbimento nelle Oloturie. *Arch. Zool.,* **1**: 1.

Kille, F. R., 1931. Induced Autotomy in Thyone. *Science,* **74**: 396.

Monticelli, Fr. Sav., 1896. Sull' autotomia delle Cucumaria planci (Br.) v. Marenz. *Rend. Accad. Lincei.* Ser. V., **5**: 231.

NOLL, F. C., 1881. (Biologische Bemerkingen). *Zool. Garten,* **22**: 171.

PEARSE, A. S., 1909. Autotomy in Holothurians. *Biol. Bull.,* **18**: 42.

SCOTT, JOHN W., 1914. Regeneration, Variation and Correlation in Thyone. *Am. Nat.,* **48**: 280.

SEMPER, C., 1868. Reisen im Archipel der Philippinen. Teil II. Bd. 1. Holothurien. Leipzig.

TORELLE, ELLEN, 1909. Regeneration in Holothuria. *Zool. Anzeig.,* **35**: 15.

A STUDY OF BACTERIAL SENSITIVITY TO ULTRAVIOLET RADIATION[1]

A. BACHEM AND M. A. DUSHKIN

(From the Department of Physiology, University of Illinois,
College of Medicine, Chicago, Illinois)

Definition of Sensitivity

Previous studies (Bachem and Dushkin, 1932) on the reactions of bacteria to ultraviolet light have demonstrated that these organisms respond with different sensitivities according to various experimental conditions. Variables, such as the wave length utilized, the age of the bacterial culture, and the temperature at which the organisms are maintained will all be reflected in the bacterial sensitivity to radiations.

The term "bacterial sensitivity" never has been well defined, nor has a uniform method been established whereby it can be measured. We have attempted to provide a reliable method of study. One may arrive at such a definition by the following experiment. By exposing bacteria seeded on an agar plate to ultraviolet rays of definite intensity per square millimeter for different exposure times, and by determining the percentage destruction of bacteria, one attains figures that can be plotted as shown in Fig. 1.

Different curves are obtained if various bacterial strains or bacteria kept at different environmental conditions are studied. Gates (1929, 1930) has shown that curves of nearly identical shape are obtained if they are plotted on a semilogarithmic scale. These curves approach straight lines over a considerable extent and are parallel to each other (Fig. 2). The tendency towards horizontal deviation from steep straight lines is most pronounced for minimal and maximal destruction. It is therefore difficult to determine exactly the energy density at which destruction of bacteria sets in and the one at which destruction is complete. The energy density at which 50 per cent of the bacteria are killed can be determined accurately by interpolation along an approximately straight line between points of lesser and greater destruction. This figure represents the average resistance of the bacteria towards ultraviolet exposure, since one-half of the bacteria exhibits more, and the other half, less resistance. We define the "average resistance" of bacteria toward ultraviolet rays as that energy required to destroy 50 per cent of the bacteria in a unit field of 1 square milli-

---

[1] This work was made possible by a research grant furnished by the American Medical Association through its Council of Physical Therapy.

meter, the energy being given as ergs. The reciprocal value of this figure represents the " average sensitivity " of the bacteria in terms of the area in square millimeters over which 1 erg must be distributed in order to destroy 50 per cent of the bacteria.

INTENSITY AND TIME

It is therefore important for us to know whether the bacterial sensitivity depends on the time during which the ultraviolet light was applied. According to Schwartzschild, identical biological effects should be expected if $i.t^n$ is constant; i representing the intensity of the rays, t the exposure time, and n an exponent usually smaller than 1. Bunsen and Roscoe, however, maintain that a constant product of $i.t$ should produce identical effects (the Schwartzschild exponent being 1 in this formula). According to the Bunsen and Roscoe law, the same energy should be required to destroy 50 per cent of the bacteria no matter whether the intensity be decreased or increased, and the exposure time prolonged or shortened correspondingly. This, when expressed in our terms, means that the average sensitivity is unaffected by intensity and

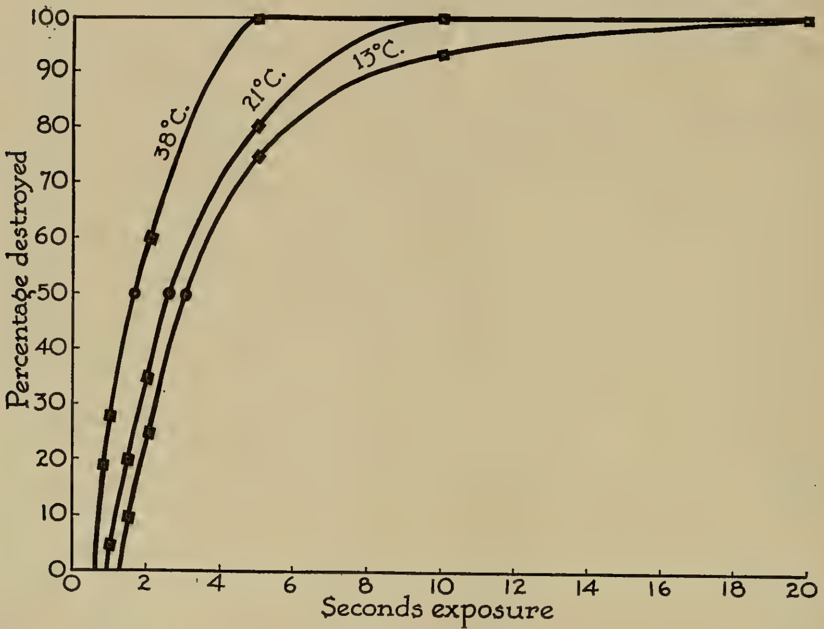


FIG. 1. Lethal effects of ultraviolet on *B. prodigiosus* plotted on linear scale. Intensity (cold quartz lamp) 4.18 ergs/(mm.^{2}sec.).

time factors. On the other hand, these corresponding changes in time and intensity are claimed by Schwartzschild to necessitate different energies in order to cause a 50 per cent bacterial destruction. The latter, paraphrased in our terms, means that the bacterial sensitivity depends upon the time distribution of the ultraviolet energy.

In order to decide between the Bunsen-Roscoe and Schwartzschild laws, we exposed agar plates seeded with *B. prodigiosus* to identical amounts of ultraviolet energy. (The source of our ultraviolet radiations in the experiments leading to the results illustrated in Figs. 3, 4, 6, and 10 was a water-cooled mercury lamp, whereas in all the other experiments, it was a cold quartz lamp. Since our photoelectric cell measures all the ultraviolet energy from 335 mμ towards shorter wave lengths, and since the spectral energy distribution differs strongly for the two sources of light, the energies are not strictly comparable). During these exposures the intensity and time factors were widely varied. The result, as indicated in Fig. 3, showed that short exposures to large intensities produced a greater biological effect and suggested that increased rather than identical applications of energy are needed at prolonged exposures to obtain identical biological results. This would demonstrate the validity of the Schwartzschild formula as applied to the bactericidal action of the ultraviolet light. As a control, we performed the same experiment but reversed the order of exposures, starting with long exposure times and low intensities. This gave us the

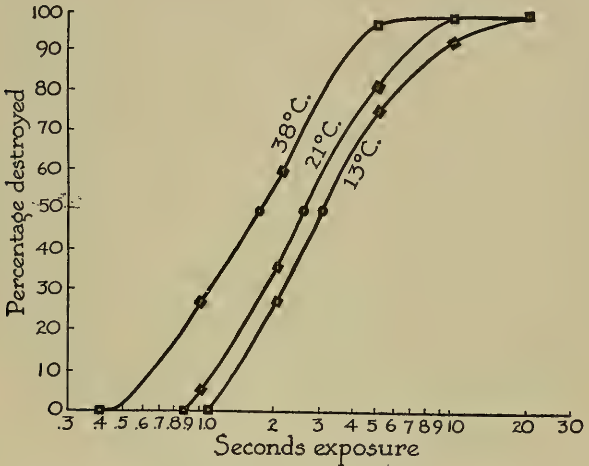


FIG. 2. Lethal effects of ultraviolet on *B. prodigiosus;* exposure times in logarithmic scale.

Intensity (cold quartz lamp) 4.18 ergs/(mm.^{2}sec.).

opposite result, namely, greatest biological effects for long exposures with small intensities (Fig. 4).

This observation evidently indicated that another important factor was involved in producing those biological responses. In order to investigate further, we exposed bacterial plates to ultraviolet light with different time intervals between plating and exposure, keeping the intensity and exposure time constant. Graphic studies of our results showed a maximum percentage of bacterial destruction occurring after

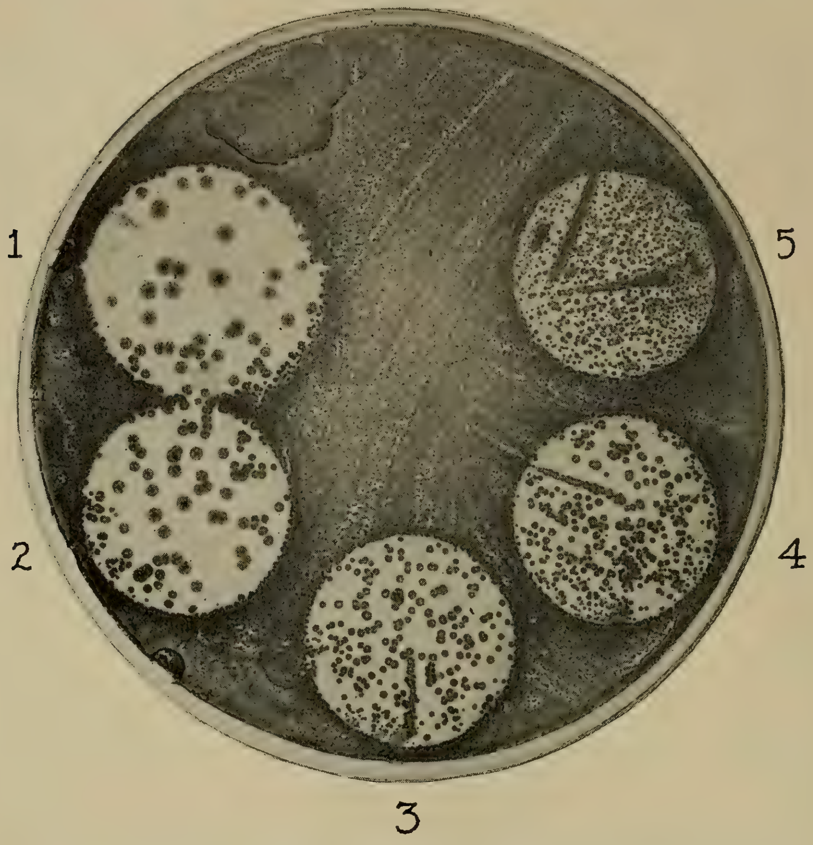


FIG. 3. Agar plate with *B. prodigiosus* exposed to constant energy (630 ergs/mm.^{2}) with variable time and intensity factors.

Exposures (started at time of maximal sensitivity):

1	2 min.	× 315	ergs/(mm.^{2}min.)
2	10 "	× 63	"
3	20 "	× 31.5	"
4	40 "	× 15.8	"
5	120 "	× 5.3	"

six hours of incubation. By varying our ultraviolet energy, we determined the energy required for a 50 per cent bacterial destruction after the various incubationary periods. These observations prove that the average resistance of the bacteria differs considerably with the incubation time; and that about 3 hours in the warmer months and approximately 6 hours in the colder months after plating, a minimum of resistance or a maximum of sensitivity exists (Fig. 5). The sensitivity varies most pronouncedly in the first few hours and again after 8 hours of incubation, whereas close to the maximum it is relatively constant

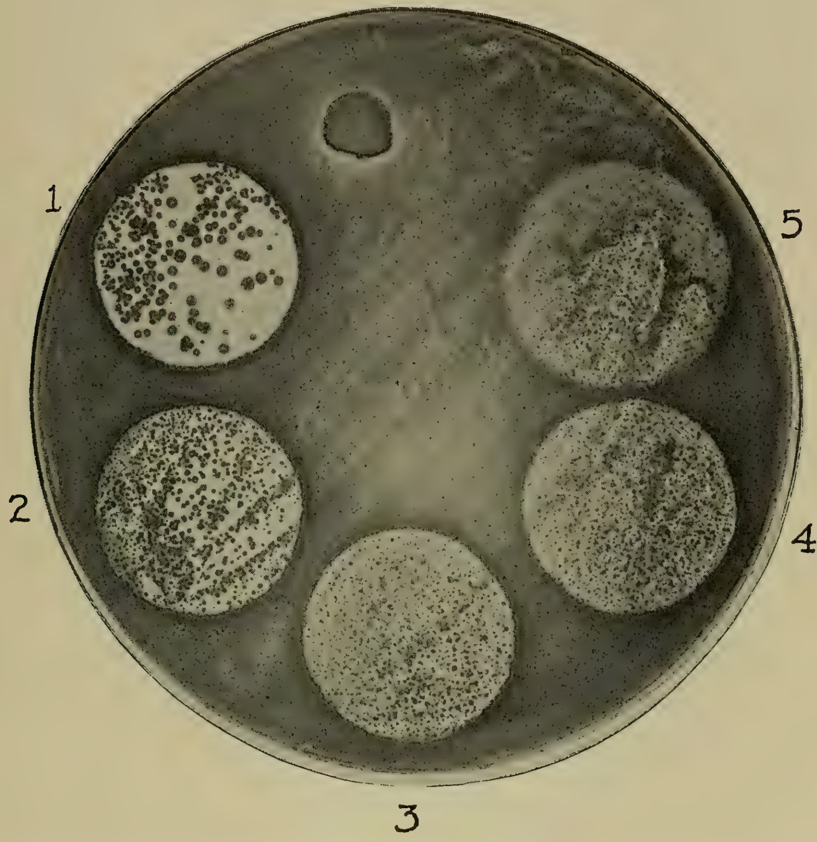


FIG. 4. Agar plate with *B. prodigiosus* exposed to constant energy (630 ergs/mm.^{2}) with variable time and intensity factors.

Exposures (started at time of maximal sensitivity):

1	120	min.	×	5.3	ergs/(mm.^{2}min.)
2	40	"	×	15.8	"
3	20	"	×	31.5	"
4	10	"	×	63	"
5	2	"	×	315	"

for a few hours. Stenstrom and Gaida (1931) claim that bacterial resistance to ultraviolet light grows with increasing incubationary periods prior to exposure. This we find to be true only after the period of maximum sensitivity has been passed. Figure 6 shows another interesting experiment in which the exposures on the left were made one-half hour after plating, and those on the right were made 6 hours after plating. The total energies applied over each exposed area were identical,

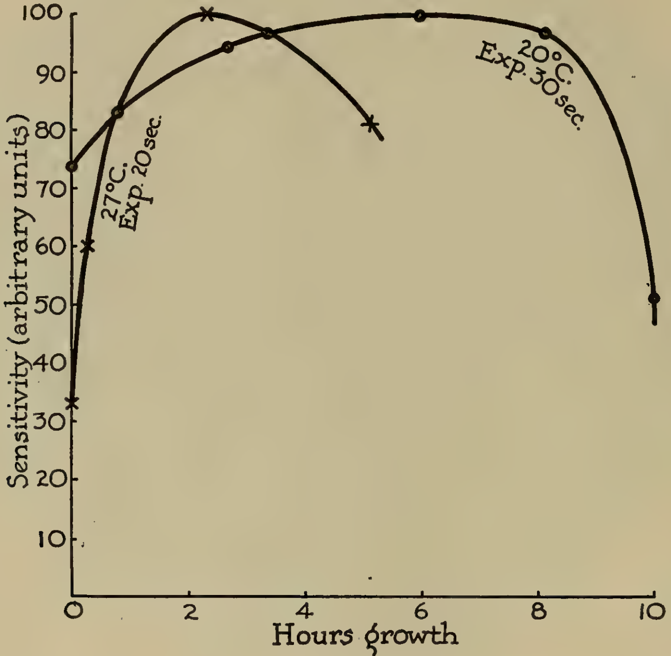


FIG. 5. Sensitivity of *B. prodigiosus* depending upon incubation temperature and time.

but three values of exposure times and intensity applications were used. The order of the exposures were such that those opposite to each other on the plate were identical. Thus the equal bacterial destruction on one side of the plate upholds the Bunsen-Roscoe law. However, the finding of increased bactericidal action after 6 hours supports our assumption that sensitivities change with different incubationary periods, and that the Bunsen-Roscoe law will hold only for those periods in which there is no change in the bacterial sensitivity.

To study further the time-sensitivity effect, we endeavored to make our observations during the period of maximal sensitivity and minimal variation of sensitivity. We exposed bacterial plates to ultraviolet light while we used different exposure times and varied the intensity by means of a rotating sector. Our results showed that when distance, light source, and temperature are kept constant, long exposures to small intensities and short exposures to great intensities yield practically identical results. Thus we find that within the range of our observations, our data supports the Bunsen-Roscoe law rather than the Schwartzschild law in its applicability to the bactericidal action of ultraviolet light. This, however, holds only for those periods in which there is no change in the bacterial sensitivity. These results conflict with the claims of Liechti (1929), who reported the Schwartzschild law to

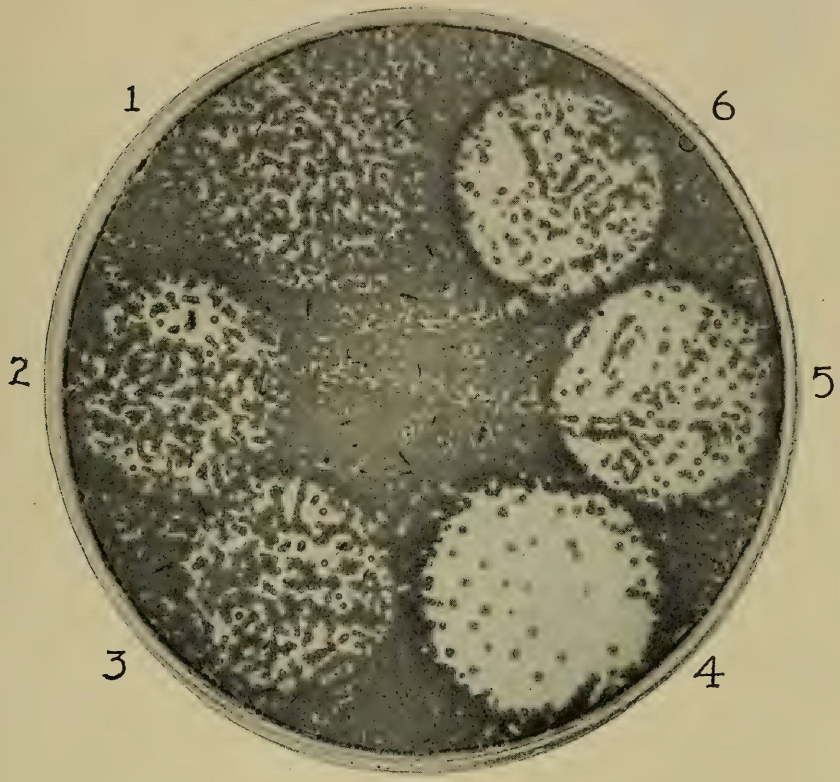


FIG. 6. Agar plate with *B. prodigiosus* exposed to constant energy (420 ergs/mm.^{2}) with variable time and intensity factors, at 2 periods of different bacterial sensitivity:

1/2 hour growth — 6 hours growth
Exposures — Exposures
1 → 1/30 min. × 12600 ergs/(mm.^{2}min.) ← 4
2 → 1/5 " × 2100 " ← 5
3 → 7 " × 60 " ← 6

be applicable to bacterial destruction by ultraviolet light; and Gates (1929), Coblentz and Fulton (1924), who deny the applicability of the Bunsen-Roscoe law but claim the validity of the Schwartzschild law. These conclusions are in agreement with those of Ehrismann and Noethling (1932), who find that the Bunsen-Roscoe law holds for changes in intensity in the ratio of 1 to 28.7 and exposure times between 1 and 100 minutes.

Temperature

Throughout the previous experiments, we noticed that during the different seasons of the year the periods of maximum sensitivity occurred after different periods of incubation. This led us to suspect that temperature exerted an influence upon the bacterial sensitivity. In order to study this possible temperature effect, we experimented at temperatures of 13°, 21°, and 38° C. *Bacillus prodigiosus* was seeded on agar plates and kept at the different temperatures stated for varying periods of time. Following this, the plates were subjected at room temperature to ultraviolet light of equal intensity but varied as to the duration of the exposure periods. The results obtained showed a lowered resistance or an increased sensitivity on the part of the bacteria at higher temperatures. In other words, in order to obtain an identical rate of destruction of the bacteria (50 per cent killed), shorter exposures of equal intensity are required at the higher temperatures (Fig. 1). The curves are more instructive when plotted on a semilogarithmic scale. The greater availability of straight lines affords easier interpolation to determine the energy required for a 50 per cent bacterial destruction. In Fig. 2, the resistance drops from 3×41.8 to 1.7×41.8 erg/mm.^{2} for a temperature rise of 13° to 38° C., or the sensitivity increases from .008 to .014 mm.^{2}/erg for the same increase in temperature.

Similar observations were reported by Howze (1926), Becquérel (1910), Mayer and Dworski (1924–25). Wiesner (1907) and Thiele and Wolfe (1907) report that the bactericidal action is hastened by a temperature increase and they further claim that ineffective rays of longer wave length are rendered bactericidal at higher temperatures. Bayne-Jones and Van der Lingen (1923) deny that the rays of a longer wave length are endowed with germicidal properties at higher temperatures, while Henri and Cernovedeanu (1910) maintain the bactericidal action of ultraviolet light to be independent of temperature.

Some authors have even gone as far as to determine a temperature coefficient for the bacterial response to ultraviolet light. Bayne-Jones and Van der Lingen determined this factor as 1.05. Gates also reports a figure close to 1.0 (about 1.1). It seems to us of great importance in determining a temperature coefficient to call attention to the following two facts:

1. Our experiments indicate that it is not the temperature during the exposure period, but the temperature of the bacterial environment prior to the exposure that influences the bacterial sensitivity to ultraviolet light. This was shown by experiments in which the plates were kept at a constant room temperature but were irradiated at — 5°, 21°, 52° C., and which revealed identical sensitivities. The sensitivity differences became manifest, when the plates were kept at different temperatures for several hours before exposure, and the exposures made at room temperature.

2. If the experiments are conducted in that way, maximal sensitivity occurs after 6 to 7 hours at low temperatures; at higher temperatures the maximum may occur after 2 or 3 hours and may be followed by a fall in sensitivity so that at the sixth or seventh hour the sensitivity is less than that shown by bacteria maintained at a lower temperature (Fig. 5). It is, therefore, evident that the temperature coefficient may vary at different periods in the experiment and that occasionally the temperature coefficient may be smaller than 1.

Growth

Our observations on the effect of temperature upon the sensitivity of bacteria to ultraviolet light lead us to the conclusion that the growth of the cultures, which depends decidedly upon incubation time and temperature, may be one of the prime factors that determine the bacterial sensitivity to ultraviolet light. We devised the following series of experiments in which the number of bacteria in a bacterial culture during definite phases of its growth could be determined, and in which we could ascertain simultaneously the degree of bacterial sensitivity to ultraviolet light. The method consisted in starting each experiment with a fresh bacterial culture. The culture was made by inoculating an Erlenmeyer flask of broth from an 18-hour culture. The flask could then be kept at a temperature of 0° C. (ice), 26° to 28° C. (room), or 37° C. (incubator); or, if so desired, we could change the maintenance temperatures. At the beginning of the experiment and at 15-minute intervals, 1 cc. of the original inoculated broth culture was diluted with a proper amount of physiological saline and seeded on two agar plates. One plate which was seeded with a higher dilution was used to determine the bacterial population of the culture at the time of exposure. The second plate, with a dilution only high enough to insure easier plate counting and yet low enough to afford uniform bacterial distribution,

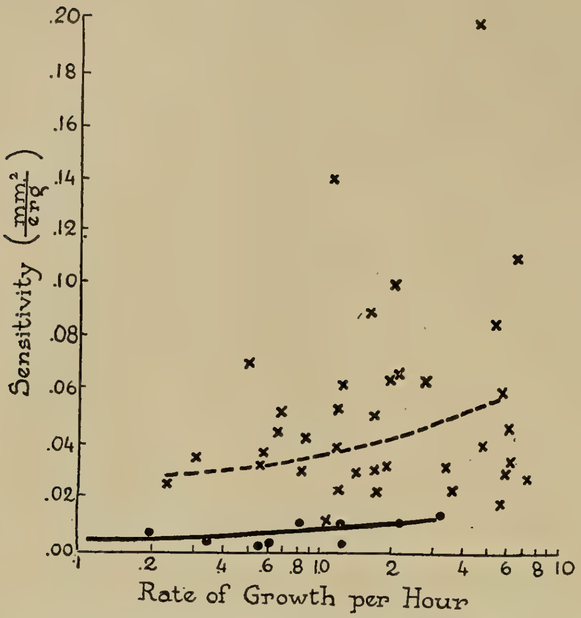


FIG. 7. Variation of bacterial sensitivity with growth rate for *B. prodigiosus,* room temperature, X---X. *Sarcina lutea,* ice and incubator temperature, •——•.

was used to determine the resistance of the culture as evidenced by the exposure required for 50 per cent destruction. The ratio of the number of bacteria at a given time to the respective number an hour before that

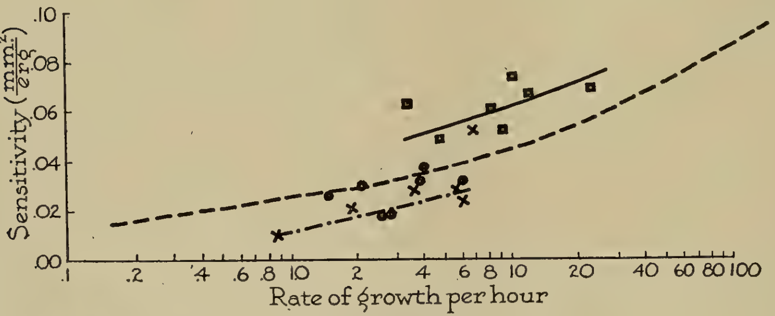


FIG. 8. Variation of bacterial sensitivity with growth rate for *B. coli* at
incubator ■ ———,
room ● - - -,
ice X—•—•— temperature.

time determined the rate of growth. In Fig. 7, we have illustrated the results obtained in studies of *B. prodigiosus* and *Sarcina lutea,* whereas Fig. 8 deals only with *B. coli.* In both graphs, one sees a rough tendency to parallelism between the rate of growth and the sensitivity of the organisms, in that as the rate of growth increases there is an increasing bacterial sensitivity to ultraviolet light. Closer studies of the graphs show, however, that the growth rate, as measured, may not be the sole determining factor in influencing the change in bacterial sensitivity. By comparing *Sarcina lutea* and *B. prodigiosus,* we find that the latter has a greater sensitivity even if organisms with identical rates of growth are compared. Furthermore, *B. coli,* when studied in three temperature ranges, showed that there is a greater sensitivity at higher temperatures although the exposures were made at times of equal growth rates. In Fig. 9, we have separated the sensitivity figures of *B. prodigiosus* for cultures with populations more and less than ten millions per cc. and have found greater sensitivities at equal growth rates in the denser cultures.

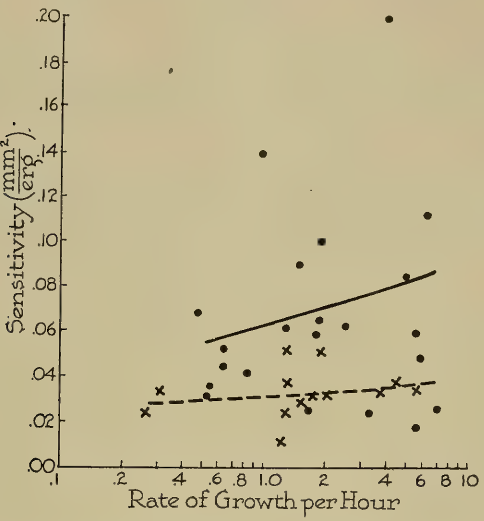


FIG. 9. Variation of bacterial sensitivity with growth rate for *B. prodigiosus* at room temperature, at different densities of population:
more than 10 million per mm.^{3} ●———
less than 10 million per mm.^{3} ×---.

Although the foregoing evidence militates against the assumption that the growth rate is the chief factor in determining the bacterial sensitivity to ultraviolet light, we feel that such may be the true relationship. This opinion is based upon the consideration that the observed cultural growth is not in any manner identical with the actual cultural growth. The observed growth is the actual growth of bacteria minus the number of organisms dying. If, therefore, the death rate is greater at higher temperatures, in denser bacterial cultures, and in cultures of *B. prodigiosus* as compared to those of *Sarcina lutea,* the actual growth rate may exceed by far the observed rate of growth and thus account for the greater bacterial sensitivity. We do not believe that the sensitivity-growth rate relationship has been as yet fully studied and are carrying on further investigations.

WAVE LENGTH

Another important factor in the consideration of bacterial sensitivity to ultraviolet light is the wave length of the bactericidal part of the spectrum.

While several authors (Ward, 1893; Bie, 1900; Barnard and Morgan, 1903; Wiesner, 1907; Bazzoni, 1914; Mackie and Von der Lingen, 1926) report bactericidal (and inhibitory) effects of visible and infrared rays, and while some authors (Bang, 1905; Bayne Jones, Coblenz and Fulton) observed a mild bactericidal action in the near-ultraviolet (300–400 mμ), most authors (Newcomer, 1917; Passow, 1919; Browning and Russ, 1919; Mashimo, 1919; Sonne, 1928; Gates, Ehrisman and Noethling) agree as to the predominant or sole bactericidal power of the ultraviolet light of wave lengths shorter than 300 mμ, and place the maximal bactericidal activity around 265 mμ.

To determine the spectral distribution of the bactericidal activity of ultraviolet light, large agar plates were seeded with bacteria (*B. prodigiosus, B. coli, Staphylococcus aureus* and *albus*). These plates were then exposed to the spectrum of the Kromayer lamp as obtained by means of a Hilger quartz spectrograph. The exposures were made at the time of minimum variation in the resistance of the organisms, which we had ascertained in our preceding experiments. We determined the relative intensity of the various spectral lines of our lamp at the Physics Department of the University of Illinois (Urbana) and found them to be as follows:

Wave length *mμ*	Intensity ergs/sec.
365	3.75
334	.32
313	3.02
302	1.23
289	.35
280	.28
265	.49
253	.67
248	.18
240	.11

Portions of the bacterial plate were exposed for different time intervals, and the relative bacterial destruction determined for the various wave lengths and times of exposure. Since these experiments were conducted at the time of maximal sensitivity and when minimal sensitivity changes occurred, the Bunsen-Roscoe law was used for our calculations. By comparing the spectral lines on the bacterial plates, the bactericidal effect of a line of a certain wave length could be matched with that of another wave length when obtained at a shorter or longer exposure time. Considering also the energy distribution throughout the spectrum, we were able to determine the bacterial sensitivity over the ultraviolet spectrum between the wave lengths of approximately 240 and 400 mμ. These results represent relative figures, since the absolute energy densities of the individual spectral lines could not be measured and the point of 50 per cent destruction could not be exactly determined. Figure 10 demonstrates graphically the results, in which the maximal relative sensitivity is called arbitrarily 100. The bacterial sensitivity starts between 313 and 302 mμ, reaches its maximum at about 260, and declines

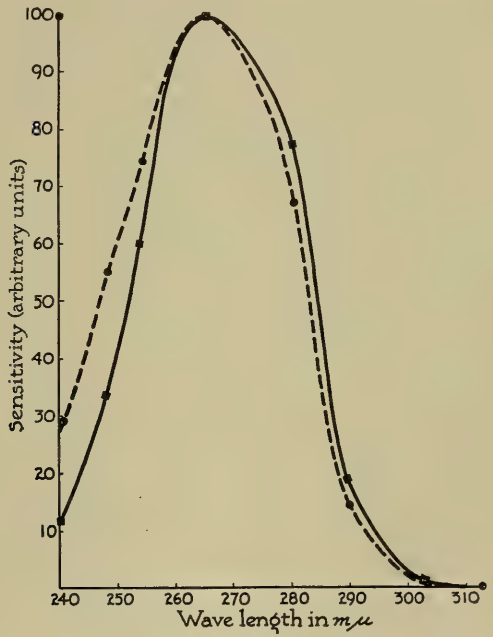


FIG. 10. Spectral sensitivity distribution (arbitrary figures) for

B. prodigiosus ■ ———
B. coli ● – – –.

towards 240 mμ, where the spectrum becomes too weak for exact investigations. Our results disagree with all those authors who maintain that the bactericidal powers of the ultraviolet increase with the decreasing wave length, but they agree with the findings of Gates, and Ehrismann and Noethling in that the bactericidal power decreases after a peak between 254–280 mμ, to a level about 240 mμ. Our findings also sustain those of Gates, and Ehrismann and Noethling in that we have not found certain wave lengths to be specific for certain organisms, but we have found that with minor variations, the spectral distribution of the bactericidal action seemed to be the same for the various organisms.

PROBLEM OF ULTRAVIOLET "STIMULATION"

Exposures of agar plates inoculated with bacteria to ultraviolet light were observed by several authors to show an increased growth of the bacterial colonies at the border of the exposed areas. This they attributed to stimulation caused by the scattered ultraviolet light of weak intensity. We use the term "stimulation" merely because it was utilized by these observers, although the phenomenon does not conform to the physiological definition of stimulation. Browning and Russ observed increased growth between two adjacent areas of destruction and

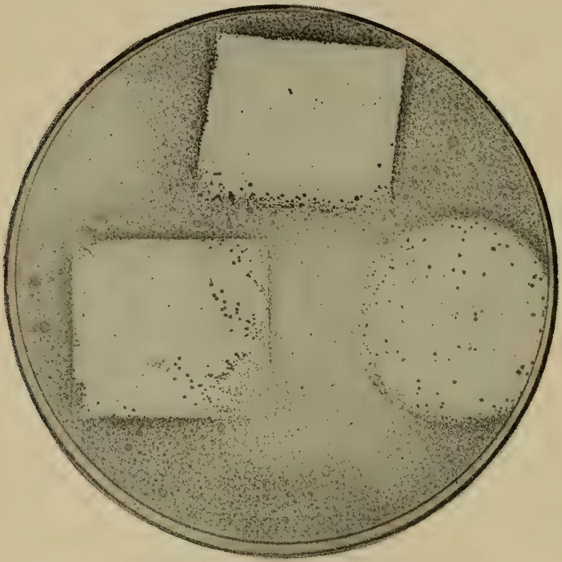


FIG. 11. Marginal growth on agar plate sprayed with *B. prodigiosus;* squares protected against spray; circle exposed to ultraviolet light.

attributed it to stimulation by ultraviolet light. Mashimo is quoted by Gates as observing this effect and as explaining it on the same basis. Coblentz and Fulton claim the stimulation of bacteria to be due either to the exposure to ultraviolet or to a larger amount of nourishment available to the cultures near the border of the exposed fields. They promised future experiments on this problem but never reported them. Gates suggests that the effects may be explained by the greater prevalence of nutritive material and more rapid disposal of toxic products of metabolism at the margin of bacterial growths; but he too failed to report any experiments along this line. In our tests we have made similar observations and assumed Gates' explanation to be the most plausible one and have conducted a few experiments which definitely ruled out direct stimulation of bacteria by ultraviolet light. Agar plates inoculated with bacteria that were exposed to sublethal doses of ultraviolet light never showed an increased growth, whereas plates exposed to visible light were inhibited for a while but later grew at a normal rate but never above normal limits. We then prepared agar plates with a definite borderline between the plated and non-plated areas in such a manner as to avoid marginal accumulation. We sprayed bacteria (*B. prodigiosus*) on the plate with two square portions of the plate protected against the spray. About these areas we find the increased mar-

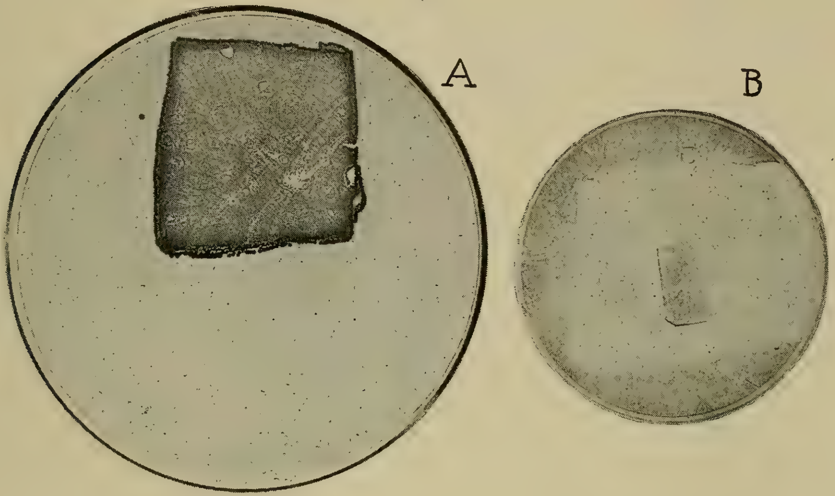


FIG. 12. Marginal growth on agar plate,
A. *B*. *prodigiosus* stamped on agar plate.
B. Agar plate with *B*. *prodigiosus,* from which stamp was cut out, and part of the stamp reinserted.

ginal growth just as well demonstrated as about the round area sterilized by ultraviolet light (Fig. 11). We then stamped bacteria on a plate by cutting out a portion of an inoculated agar plate and holding it against a sterile plate (Fig. 12, *A*). At the margin of the bacterial area that had been stamped on, we again found an incresed growth; nor was any increased growth seen on the margin of the stamp, a portion of which is seen in Fig. 12, *B*. This is explainable on the basis that in these latter two instances there were no media from which extra nutritive material could be obtained and towards which toxic products of metabolism could be dissipated. From these experiments we come to the conclusion that wherever room is available for diffusion of nutritional material and toxic products, increased growth occurs; and where the field is limited to the culture, no increased growth will occur. Therefore, the phenomenon is not characteristic for exposures to ultraviolet light and hence is not the result of stimulation of the bacteria by the ultraviolet rays.

CONCLUSIONS

1. The "average resistance" of the bacteria towards ultraviolet light is the energy required to destroy 50 per cent of the bacteria in a given field; the energy being given as ergs per mm.^{2} The reciprocal value of this figure is the "average sensitivity" of the bacteria.
2. The Bunsen-Roscoe law rather than the Schwartzschild law is applicable to the bactericidal action of ultraviolet light, but is true only for these periods in which there is no change in the bacterial sensitivity.
3. The average sensitivity is influenced by the temperature at which the exposures are made. The temperature coefficient depends upon so many variables that it cannot be represented by a single value.
4. The average sensitivity varies with the incubation time; the rate of bacterial growth apparently being the most important factor in causing the variation.
5. Bactericidal action begins at 302 mμ, increases towards 265 mμ, and decreases towards 240 mμ. The spectral distribution is not specific for any one of the organisms studied.
6. Stimulation of growth was not observed to result from the action of ultraviolet light on bacteria.

We are greatly indebted to Dr. L. Arnold, Professor of Bacteriology, for his advice throughout our bacteriological studies.

BIBLIOGRAPHY

BACHEM, A., AND M. A. DUSHKIN, 1932. *Proc. Soc. Exper. Biol. and Med.*, **30**: 700.
BANG, S., 1905. *Mitt. aus Finsen's Med. Lysinstitut,* **9**: 164.

Barnard, J. E., and H. de R. Morgan, 1903. *Brit. Med. Jour.,* **2**: 1269.

Bayne-Jones, S., and J. S. Van der Lingen, 1923. *Johns Hopkins Hospital Bulletin,* **34**: 11.

Bazzoni, C. B., 1914. *Am. Jour. Public Health,* **4**: 975.

Becquérel, P., 1910. *Comp. Rend. Acad. Sci.,* **151**: 86.

Bie, W., 1900. *Mitteil. aus Finsen's Med. Lysinstitut,* Leipzig, p. 78.

Browning, C. H., and S. Russ, 1917. *Proc. Roy. Soc., London, B,* **90**: 33.

Cernovedeanu, P., and V. Henri, 1910. *Comp. Rend. Acad. Sci.,* **150**: 52.

Coblentz, W. W., and H. R. Fulton, 1924. *Sci. Papers, Bureau Standards,* **19**: No. 495.

Ehrismann, O., and W. Noethling, 1932. *Zeitschr. f. Hyg. u. Infectionskrankh.,* **113**: 597.

Gates, F. L., 1929. *Jour. Gen. Physiol.,* **13**: 231, 249.

Gates, F. L., 1930. *Jour. Gen. Physiol.,* **14**: 31.

Howze, H. H., 1926. *Am. Rev. Tuberculosis,* **13**: 470.

Liechti, A., 1929. *Strahlentherapie,* **33**: 1.

Mackie and Van der Lingen, 1926. Quoted from E. Mayer.

Mashimo, T., 1919. *Mem. Coll. Sci., Kyoto Imperial University,* **4**: 1.

Mayer, E., 1926. Clinical Application of Sunlight and Artificial Radiation.

Mayer, E., and M. Dworski, 1924. *Am. Rev. Tuberculosis,* **10**: 166.

Newcomer, H. S., 1917. *Jour. Exper. Med.,* **26**: 841.

Passow, A., 1919. *Med. Klin.,* **15**: 1307.

Sonne, C., 1928. *Strahlentherapie,* **28**: 45.

Stenstrom, W., and J. B. Gaida, 1931. *Proc. Soc. Exper. Biol. and Med.,* **28**: No. 9, p. 898.

Thiele, H., and K. Wolf, 1907. *Arch. f. Hyg.,* **60**: 29.

Ward, H. M., 1893. *Proc. Roy. Soc.,* **54**: 472.

Wiesner, R., 1907. *Arch. f. Hyg.,* **61**: 1.

STRUCTURE OF THE CHROMOSOMES IN THE SALIVARY GLAND CELLS IN SCIARA (DIPTERA)

W. L. DOYLE AND C. W. METZ

(*From the Zoölogical Laboratory of the Johns Hopkins University and the Department of Embryology of the Carnegie Institution of Washington*)

Recent studies on the chromosomes of the larval salivary glands of Diptera, initiated by the work of Heitz and Bauer (1933) and of Painter (1934), have served to emphasize the great usefulness of these structures for investigations on chromosome organization. One such line of investigation, as Painter has intimated, should aim at an identification of individual genes and at an interpretation of structures visible in " ordinary " chromosomes in terms of the much larger structures to be seen in the giant salivary gland chromosomes. Contributions have already been made to this subject by Koltzoff (1934), Bridges (1935), Metz and Gay (1934), and Muller and Prokofyeva (1935), and the present account is designed for the same purpose.

In the work reported here attention has been devoted especially to a study of the chromosomes in the living condition and to the effects of different reagents upon them. Particular attention has been paid to the effects of acetic acid because the interpretations of earlier investigators have been based largely on study of material fixed in this acid. *Sciara* has been used for study because its chromosomes are unusually large and favorable for the purpose.

Larvæ of the fungus-gnats *Sciara coprophila* (Lint.) and *S. ocellaris* (Comst.) obtained from cultures maintained in the laboratory were used. Fairly clean larvæ were washed in Ringer's solution, dried on filter paper, and placed on a slide for dissection under a Greenough binocular. In each dissection the head was cut off by means of sharp needles, the salivary gland cut free and immediately placed in isotonic salt solution on another slide and covered with a cover-slip supported by vaseline and glass threads. During dissection some of the larvæ were immersed in salt solution isotonic with the gland cells, others were exposed to air so that the gland cells were immersed in larval body fluid until transferred to the second slide, others were immersed in mineral oil during dissection and studied on the same slide so that the glands were continuously in larval body fluid which had not been exposed to air. Precautions were taken to prevent evaporation with attendant concentration of salts in the medium. The method of insuring isotonicity of saline solutions is described below.

Solutions, the effect of which it was desired to test, were run under the coverslip at one side and the previous solution simultaneously removed at the other side by means of filter paper. The vaseline and glass threads prevented compression of the glands between the coverslip and slide during mounting and changing of solutions. Objectives of sufficient working distance to prevent pressure on the coverslip were used (3 mm. n. a. 1.4 oil immersion). Measurements were made with a calibrated ocular micrometer.

OBSERVATIONS

Structure of the Gland

The salivary gland of the larva of *Sciara* varies in size according to the size and age of the larva. The smallest gland here considered, however, has the same number (approximately 150) and arrangement of cells as the largest. The difference in size is dependent entirely on the growth of the cells.

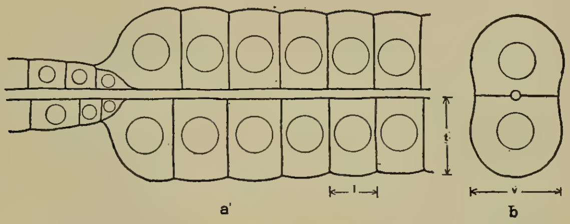


FIG. 1. Outlines of typical side view (*a*) and cross-section (*b*) of salivary gland of *Sciara*. *L*, longitudinal axis of cell; *t*, transverse axis; *v*, vertical axis.

The gland may be divided for convenience into three portions, the duct (not glandular and not included in cell counts), the 28–32 cells nearest the duct (proximal portion), and the remaining 120 cells surrounding the convoluted portion of the lumen (convoluted portion, Figs. 1 and 2). The arrangement of the cells in the proximal portion is such that when the gland is lying flat on the slide all the details in any cell can be seen, since the gland is only one cell thick when viewed in this position (Fig. 1*a*). In normal living glands mounted in larval fluid surrounded by mineral oil the nuclei are spherical and the transverse, longitudinal, and vertical dimensions of a cell in any one position in the gland (Fig. 1) have a fairly constant ratio to one another regardless of the size of the gland. A small gland has the same proportions as a large one.

In glands mounted in mammalian Ringer's solution the dimensions of the cells immediately change so that they no longer bear the same ratio to each other and the nuclei are no longer spherical. It is apparent that such a solution is hypotonic and that the cells are swelled. Owing to the arrangement of the cells in the gland and the relative inelasticity of the wall of the lumen, the swelling is more pronounced along the transverse and vertical axes than along the longitudinal axis of the gland.

Similarly, in glands mounted in hypertonic salt solutions the cells and nuclei immediately shrink and the shrinkage is more pronounced along the same axes. Having ascertained the normal ratio of transverse to longitudinal axes and having found that the normal nucleus is spherical, it is necessary only to measure these axes of the cell or nucleus to determine whether or not the solution in which they are mounted is hypo-, hyper- or isotonic with the cell.

Structure of the Nucleus

When observed in an isotonic salt solution, the nuclei of an active (not pupating) larva of *Sciara* show no indication of chromosomes or of the chromatic segments (discs, bands) characteristically seen in aceto-carmine preparations. Instead, the nuclear membrane appears to enclose a homogeneous fluid containing several small granular regions. This granular material is arranged in thin layers which serve to outline optically empty contorted cylinders. It represents the nucleolus or chromocenter [1] of the nucleus and comprises a relatively small percentage (not over 10 per cent) of the volume of the nucleus. The convoluted cylinders (presently shown to be the chromosomes) and the granular material appear to fill the nucleus completely; there is no visible extra-chromosomal nuclear fluid.

The striking disparity between this condition and that seen in aceto-carmine preparations is immediately apparent.

Effects of Acetic Acid

If living glands from normal larvæ are examined under high magnification while the isotonic salt solution surrounding the glands is being replaced by aceto-carmine,[2] one observes that in the space of one to two

---

[1] When fixed in formalin (basic fixation image) and stained in ferric hæmatoxylin the chromocenter granules, which are of various sizes, are deeply stained, whereas the chromosomes are only faintly stained. This indicates that the chromocenter is like a plasmosome nucleolus.

[2] Since carmine renders the cytoplasm opaque, whereas the 45 per cent acetic acid alone does not, the structure of the chromosomes in these preparations is more clearly visible without the stain; and since the fixation images of both aceto-carmine and 45 per cent acetic acid are identical, the acid alone was used in subsequent observations.

seconds the cells swell (10 per cent transversely), the mitochondria disappear, the nuclei swell, the nucleolar granules dissolve, the optically homogeneous cylinders become translucent, segments appear in them, they writhe about in the nucleus and they shrink in volume (50 to 70 per cent in diameter) so that they now comprise less than half the volume of the nucleus. As segments appear in the cylinders, it is sometimes observed that during the shrinkage portions of adjacent cylinders move in opposite directions along their long axes. This occurs when a portion of one is rather firmly fixed to the wall of the nucleus on one side and a portion of the other is attached to the opposite side of the nuclear wall. In other nuclei the cylinders become opaque and contracted in such a manner that all the chromosomes are found in a single clump with little fluid between them. In such chromosomes it is not possible to distinguish definite segments.

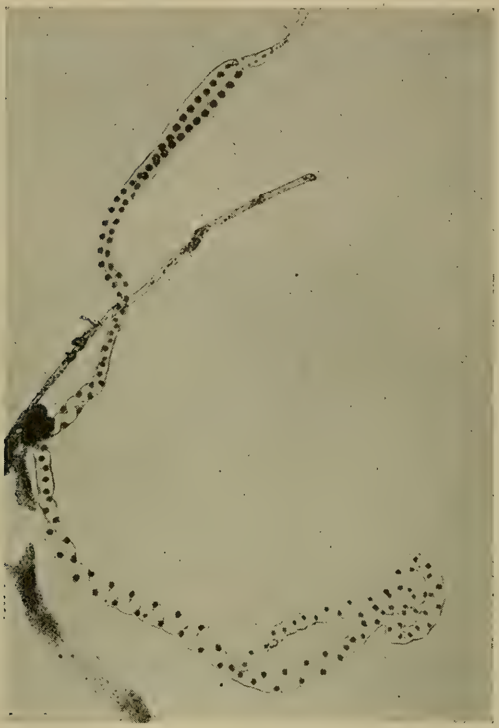


FIG. 2. Photograph of one entire salivary gland showing arrangement of nuclei in the different regions of the gland. Feulgen preparation. × 54.

During fixation the changes in morphology of the cellular structure occur with such rapidity that only a few details can be observed in process in any one preparation. For this reason, the results of repeated observations are generalized in the above description. Furthermore, owing to the magnitude of the changes and the speed and distance the chromosomes move, it is not possible to observe visually the finer details in process of changing. In order to overcome this difficulty, various combinations of reagents were employed as noted below.

Effects of Formalin

Ten per cent formalin in an otherwise isotonic salt solution was used to replace the medium in which living glands were mounted. With such fixation the drastic changes produced by acetic acid are absent. When the process is observed under the microscope the first indication of any change is a slight opacity of the cytoplasm, followed by shrinkage (9 per cent transversely) of the cell and shrinkage (8 per cent transversely) of the nucleus, with no measurable change in diameter of the chromosomes. The chromosomes are no longer transparent but are translucent, yet still apparently as homogeneous as in living material. The mitochondria in the cytoplasm and the nucleolar granules are still visible. Such preparations were examined at intervals over periods of from five minutes to 72 hours and the following changes were noted. The cells, the nuclei, and especially the chromosomes shrink very gradually when left in formalin, so that after 72 hours the chromosomes occupy approximately 70 per cent of the nuclear volume. Since dehydration of such material in alcohol results in further shrinkage of all components, it would appear that formalin fixation results in incomplete coagulation of the colloids, i.e., in incomplete entmischung (elution) of the aqueous and non-aqueous phases.

Effects of Acetic Acid after Fixation in Formalin

When a formalin solution (which has been allowed to act for fifteen minutes) is replaced by acetic acid, a rapid change takes place in all the components of the gland. The cells swell (20 per cent transverse measurement), the chondriosomes disappear, the nuclei swell to original size, the nucleolar granules disappear, and the chromosomes shrink slightly in width in the achromatic regions (i.e. achromatic segments)[3] and tend to increase slightly in length. Some of the chromatic segments which appear as thin smooth lines in formalin become zig-zag lines in acetic acid and vesicles appear in both the chromatic and the achromatic segments. It is improbable, then, that these vesicles represent genes around which chromatin has condensed as has been suggested by Bridges (1935).

---

[3] The terms chromatic segments and achromatic segments are used here to designate respectively the " discs " or " bands " and the achromatic regions between them.

Effects of Osmic Acid

Isotonic salt solutions surrounding fresh glands were replaced by osmic acid made up in two ways: one in isotonic saline, the other in distilled water. There is a notable difference between the effects of the two solutions. Osmic acid in distilled water causes two observable changes in the chromosomes. The first is a swelling, during which the chromosomes appear to become more homogeneous, followed in about one-half second by a granulation which takes place without appreciable change in proportions of either achromatic or chromatic segments. In osmic acid solutions made up in isotonic saline, the preliminary swelling does not occur. The details of the granulation are essentially the same in both instances. As the chromosomes granulate they do not change conspicuously in diameter and the distance between chromatic segments does not change appreciably. The material in both the chromatic and the achromatic segments is precipitated in a finely granular coagulum. The nucleolar granules are faithfully preserved. Subsequent treatment with acetic acid causes no appreciable changes in proportions of chromatic and achromatic segments.

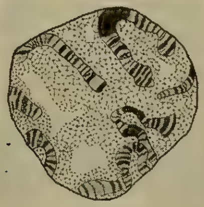


FIG. 3. Camera outlines of nucleus and chromosomes of salivary gland cell fixed in Gilson and sectioned, showing extra-chromosomal coagulum. 2 mm. objective. 15 × oc.

Effects of Mercuric Chloride

Fixation in mercuric chloride results in violent shrinkage of the nucleus. One feature of the fixation image is of special interest at this point. The fluid lost from the chromosomes as they shrink is coagulated by the $HgCl_2$ (Fig. 3). This shows that during shrinkage of the chromosomes organic material as well as water and possibly salts is extruded from the chromosome coagulum. The nature of this material has not yet been ascertained.

Effects of Varying the Concentration of Saline Solutions

Glands were mounted in isotonic saline solutions and the same saline constituents in various concentrations were substituted for the isotonic concentration. As the concentration of salts is increased, the cell decreases in size (in one to two seconds), the nucleus shrinks, and the diameter of the chromosomes decreases (in four to five seconds), the thickness of the chromatic segments or discs increases, the distance between them decreases, additional chromatic segments become apparent, and those present become more distinct. Apparently some chromatic segments which were originally in the form of thin lines become first zig-zag and finally rows of granules as the salt concentration is increased. The size of the nucleus in any of these solutions is directly correlated with, but it is not arithmetically proportional to, the diameter of the chromosomes. Since the nuclear membrane remains smooth it would appear to be elastic.

Essentially the same changes occur if glycerine is substituted for some of the water in an isotonic saline solution. This indicates that the shrinkage of the chromosomes is an osmotic rather than salting out effect of the higher salt concentrations.

If the hypertonic salt solution is now replaced by the original isotonic saline (allowing sufficient time for washing out diffusing salts) all constituents return to a volume which (within the limits of error of measurement) is the same as when measured in the original isotonic solution. The chromosomes and nucleolar granules again appear to fill the nucleus completely and the chromatic segments disappear so that the chromosomes are again optically homogeneous (Table I, *b*).

If isotonic salt solution is replaced by hypotonic solution, the cells, nuclei, and chromosomes all increase in volume. The chromosomes remain homogeneous.[4] Upon replacing hypotonic solutions with an isotonic solution approximately the original volume and appearances are regained.

When this process of varying the osmotic pressure is repeated several times on one preparation either by lowering or by raising the salt concentration and returning to isotonic solutions, or both; it is observed that when finally returned to isotonic solutions the volumes of the components are all less than when originally measured (Table I, *a, c*). In addition, segments are visible where the chromosome originally appeared to be homogeneous. The general appearance resembles that of the preparation when originally placed in slightly hypertonic solution. This is equally true of preparations subjected only to hypo- and isotonic solutions. It seems evident that the contraction and expansion of the chromosome colloids attendant on variations in the osmotic pressure has resulted in a leaching out of the original osmotically active substances and a substitution of others in their places. Under these changed conditions, the various components no longer regain their normal degree of hydration in a solution originally isotonic. It would appear that the protoplasmic colloid has undergone a certain degree of syneresis.[5] If this is true, the same effects should be producible by maintaining a constant osmotic pressure and varying the hydrogen-ion concentration of the saline solutions. As the isoelectric point of the protein constituents is approached, the syneresis should be more pronounced. Preliminary experiments indicate that this is true.

---

[4] Decrease in osmotic pressure of the mounting medium results in an increase in distance between chromatic segments in a chromosome. As chromatic segments disappear (as the chromosomes swell and become optically homogeneous), they are moving farther apart from each other and are becoming thinner. There is no evidence that the chromatic segments fuse.

TABLE I

	Minutes after dissection	Strength of solution *	Diameters of nucleus	
			Long.	Trans.
(*a*)	0	0.9	28	29
	3	0.7	26	21
	7	0.9	28	28
	10	0.7	28	20
	15	0.9	27	25
	19	0.7	25	19
	25	0.9	25	22
(*b*)	0	0.9	29	28
	3	0.7	28	20
	6	0.9	29	28
	9	0.88	28	28
	12	0.7	28	20
	15	0.9	28	28
(*c*)	0	0.9	35	36
	5	1.1	35	42
	20	0.9	33	30
	25	1.1	33	38
	35	0.9	32	28
	40	1.1	33	36
	45	0.9	32	28

* 0.9 is isotonic, 1.1 is hypotonic, 0.7 is hypertonic.

---

[5] When nuclei have been shrunk by hypertonic solutions the cytoplasm immediately surrounding the nuclei is more transparent than that of the rest of the cell. This halo then persists even when solutions of normal or hypotonic osmotic value are substituted for the hypertonic medium. This is taken to indicate that specific fluid materials lost in shrinkage of the nucleus are not completely regained when the nucleus swells but rather that they are replaced by others.

Effects of Light

Chromosomes in preparations in isotonic saline solutions exposed to intense illumination undergo irreversible changes which are essentially similar in nature to those caused by acetic acid. In visible light these changes take place gradually; in ultra-violet radiation (.275 $\mu\mu$ from a cadmium spark) they occur very rapidly, the actual length of time depending on the intensity (less than one minute in our experiments).

Effects of Larval Age

The above observations are typical of experiments on large active larvæ. Glands of young larvæ, prepupæ, and early pupæ behave in essentially the same manner with one outstanding difference. (It is evident that structures near the limit of resolution of the microscope in the larger specimens will be more clearly defined than homologous structures in the smaller specimens. In full consideration of this fact the following observations are reported). The same degree of increase in osmotic pressure of the mounting medium results in a greater proportional change in the chromosomes in older specimens than in younger ones. In other words, it requires less dehydrating force to produce the dehydrated image in glands of old specimens (prepupæ, etc.) than in glands of young ones (early larvæ). Similarly, for the same number of changes in osmotic pressure of the mounting medium, a greater degree of histeresis occurs in the older specimens than in the younger ones. That is, the younger specimens appear to recover more completely and for a greater number of times when returned to an isotonic solution from an anisotonic one.

Discussion and Conclusions

Chromosome Sheath

As one observes the first appearance of segments in homogeneous chromosomes, it becomes evident that the segments are equal in diameter to the diameter of the homogeneous cylinders which make up the nucleus. The limiting membrane of the cylinders is a very delicate scarcely differentiated structure. If a differentiated layer surrounds the chromosomes separating them from one another (chromosome sheath) in these nuclei, it is so thin as to be unresolvable as a layer but appears as a fine single line in our preparations.[6]

---

[6] It seems probable that the extrusion and absorption of material by the chromosomes may be related to the chromosome sheath. In other words, the differentiated material seen to surround chromosomes at certain stages appears to be a result of the extrusion of material from the chromosome. Whether this ex-

General Chromosome Structure

From these observations it seems apparent that the normal living structure of the chromosomes in these cells is that of a delicately poised colloidal gel of complex constitution. The behavior of this gel when subjected to various experimental fluids indicates that variations in osmotic pressure, acidity, and specific ions in the fluids result in various morphological images. Almost all experimental solutions result in loss of water from the gel and in an aggregation or precipitation of the dispersed phase to form an artificial structure or matrix of varying appearance. The behavior of this material indicates that it is proteic in nature. The fineness or coarseness of the reticulum and the consequent size of inter-reticular spaces is dependent on the nature of the coagulant employed and the manner in which it is used. The appearance and disappearance of the segments in the chromosomes is dependent on the changes in indices of refraction of chromatic and achromatic segments as they are variously hydrated and dehydrated, and not upon a separation and fusion of the chromatic segments. That is, the chromatic segments are always present as differentiated structures, although they are not always visible. The number and size of the vesicles in the chromatic and achromatic segments appearing during fixation of the chromosomes is dependent in part on the nature and method of application of the coagulant.

Whereas aceto-carmine preparations give excellent images of the presence and arrangement of segments in the chromosomes, the finer details of structure to be seen in them appear to be artifacts.[7]

BIBLIOGRAPHY

Bridges, C. B., 1935. *Am. Nat.,* **69**: 58.
Heitz, E., and H. Bauer, 1933. *Zeitschr. f. Zellforsch. u. mikr. Anat.,* **17**: 67.
Koltzoff, N., 1934. Science, **80**: 312.
Metz, C. W., and E. H. Gay, 1934. *Science,* **80**: 595.
Metz, C. W., and E. H. Gay, 1934. *Proc. Nat. Acad. Sci.,* **20**: 617.
Muller, H. S., and A. A. Prokofyeva, 1935. *Proc. Nat. Acad. Sci.,* **21**: 16.
Painter, T. S., 1934. *Genetics,* **19**: 175.

---

truded material is always formed by the coagulating action of fixatives or may sometimes be produced normally by the chromosome at various stages of the division cycle remains to be investigated.

[7] See also Metz and Gay (1934) for additional evidence on this point,—particularly differences due to methods of dissection or treatment before fixation in acetocarmine.

OBSERVATIONS ON THE FEEDING OF DIFFLUGIA, PONTIGULASIA AND LESQUEREUSIA

A. B. STUMP

(From the Miller School of Biology, University of Virginia)

Introduction

In October, 1930, a few *Lesquereusia spiralis* (Ehrenberg) were seen attached to *Spirogyra.* Careful observation showed that these animals were feeding on the algæ in a manner similar to that described for *Vampyrella,* but apparently not described for the Testacea. In April, 1931, *Pontigulasia vas* (Leidy) Schouteden and *Difflugia constricta* (Ehrenberg) were seen feeding on green filamentous algæ in the same way. During the next three years experiments were made to ascertain how widespread this feeding habit was among the Testacea and to note any differences in the process which might appear in the various species. This paper has for its purpose a description of the method by which *Lesquereusia spiralis, Pontigulasia vas,* and a number of the *Difflugia* remove the cell contents of *Spirogyra* and other filamentous green algæ for food.

Full acknowledgment and thanks are given to Dr. B. D. Reynolds for suggestions and advice in directing this work.

Material and Methods

The Testacea used in this study were collected from a number of ponds in the vicinity of Charlottesville, Virginia. After isolating them in small dishes the animals were identified. Little difficulty was experienced in identifying the *Lesquereusia* and *Difflugia.* The *Pontigulasia,* on the other hand, were for a long time considered to be *Difflugia oblonga* and it was only when these animals were sectioned in a later study that this mistake was discovered. The confusion arose from the fact that the *Pontigulasia vas* in this locality fail to show a deep constriction between the neck and the fundus of the shell as described by Leidy (1879), Penard (1902), Cash (1909), and Kudo (1931) for this form. Moreover, the internal diaphragm is easily overlooked in toto mounts.

As the methods used were essentially the same for all the Protozoa experimented with, only those for *Lesquereusia* will be described here.

Cultures were kept in small Stender dishes or Petri dishes. One or two *Lesquereusia,* placed with *Spirogyra* in tap or spring water, were used to start cultures which lasted from four to six weeks.

Observations on the *Lesquereusia, Pontigulasia,* and *Difflugia* were made with a wide field binocular or a microscope using the 16 mm. objective. For a more minute study to show their action in feeding on the algæ, the hanging drop method was used, making it possible to use both the 4 mm. and oil immersion objectives. Drawings were made by camera lucida from these hanging drop cultures.

A number of permanent slides were made showing these Testacea feeding on *Spirogyra.* Schaudinn's fixing fluid and Delafield's hæmatoxylin stain were used for most of this material. A few sections were made of *Lesquereusia* feeding on *Spirogyra.* These slides were made by the usual method, the sectioning was at 12 μ. Both camera lucida drawings and photo-micrographs were made from permanent slides.

Observations

Lesquereusia spiralis was observed to attack *Spirogyra* and devour the cell contents in the following manner: After locating the alga the rhizopod would pull itself up to the filament so that the mouth of the shell was in contact with the cell wall. If the animal happened to be attacking near the end of a filament it would often attach itself to the end of the terminal cell (Fig. 2, Pl. I). The cellulose wall was rarely drawn into the mouth of the shell. Shortly after reaching its final position all movement ceased and the pseudopods were withdrawn. In twenty to thirty minutes from the time the last pseudopods were seen, the *Spirogyra* cell would begin to show plasmolysis. The protoplast first pulls away from the transverse septa and rather slowly collapses into a mass along the axis of the cell. At this time there is often a bending or breaking of the filament. This is similar to the effect on *Spirogyra* caused by *Vampyrella* as described by Lloyd (1929) and others. There is, however, no sudden snap as caused by *Vampyrella.*

After plasmolysis the protoplast gradually moves toward the point of attachment. In a short time the chloroplasts can be seen streaming through a hole in the cell wall into the animal. This process continues steadily until the *Spirogyra* cell is empty (Figs. 1, 2, 3, and 4, Pl. I). It takes from twenty to thirty minutes for the cell to be emptied after the first plasmolysis.

Not until the *Spirogyra* cells have been almost emptied will any pseudopods be visible. *Lesquereusia* has been observed to extend pseudopods within the cell and tear loose portions of the chloroplasts which had remained attached to the cell wall. This is somewhat different from *Vampyrella,* which, according to Lloyd (1929), has never been observed to extend pseudopods within the cell. Having emptied one cell the animal loosens its grip on the filament and moves on to another cell where the same process is repeated. About one hour is required to devour one cell. One *Lesquereusia* has been observed to empty seven cells in nine hours. The time taken for the last two or three cells was considerably more than usual, the animal appearing to be sluggish with excess food. The cells of *Œdogonium* and *Zygnema* were attacked and emptied in the same way. When *Mougeotia* was attacked at the end of a filament the whole series of cells would be taken in through the mouth and down into the fundus of the shell. In this case apparently the entire cell was ingested,—both wall and contents. The *Lesquereusia* would continue to ingest the cells of the filament until it was entirely consumed.

The feeding of *Pontigulasia vas* and *Difflugia lobostoma* is so similar with regard to these algæ that a detailed account is unnecessary here.

Difflugia constricta also exhibited substantially the same feeding actions as the other three thecamœbæ. Observations with this species involved *Zygnema* and *Stigeoclonium. Spirogyra* was not eaten by this form, possibly because the cell walls were too tough.

The method by which the algæ cells were entered was difficult to ascertain, in spite of the fact that the process was observed a number of times. This difficulty arose from the fact that when attached to the filament the animal's body cut off the view of the cell wall immediately in front of the mouth. A study of the emptied cells revealed that the cell walls were torn, as rents from 10 to 25 microns long were evident. It seems probable, however, that there was some softening of the cellulose wall before any tearing or puncturing took place. Sections of

---

EXPLANATION OF PLATE I

(Figures based on photographs)

FIG. 1. *Lesquereusia spiralis* devouring the cell contents of *Spirogyra.* Two pieces of the chloroplasts are entering the mouth of the shell. × 120.

FIG. 2. *Lesquereusia spiralis* (side view) removing the contents of a *Spirogyra* cell. The chloroplasts are seen as the dark masses extending out from the mouth of the specimen. × 120.

FIG. 3. *Pontigulasia vas* attached to the terminal cell of *Spirogyra* filament. The cell is almost empty. × 120.

FIG. 4. *Pontigulasia vas.* The *Spirogyra* cell is plasmolysed and the protoplast just beginning to be drawn in. × 120.

FIG. 5. Section of *Lesquereusia spiralis* and *Spirogyra* filament showing entrance into the algal cell. × 500.

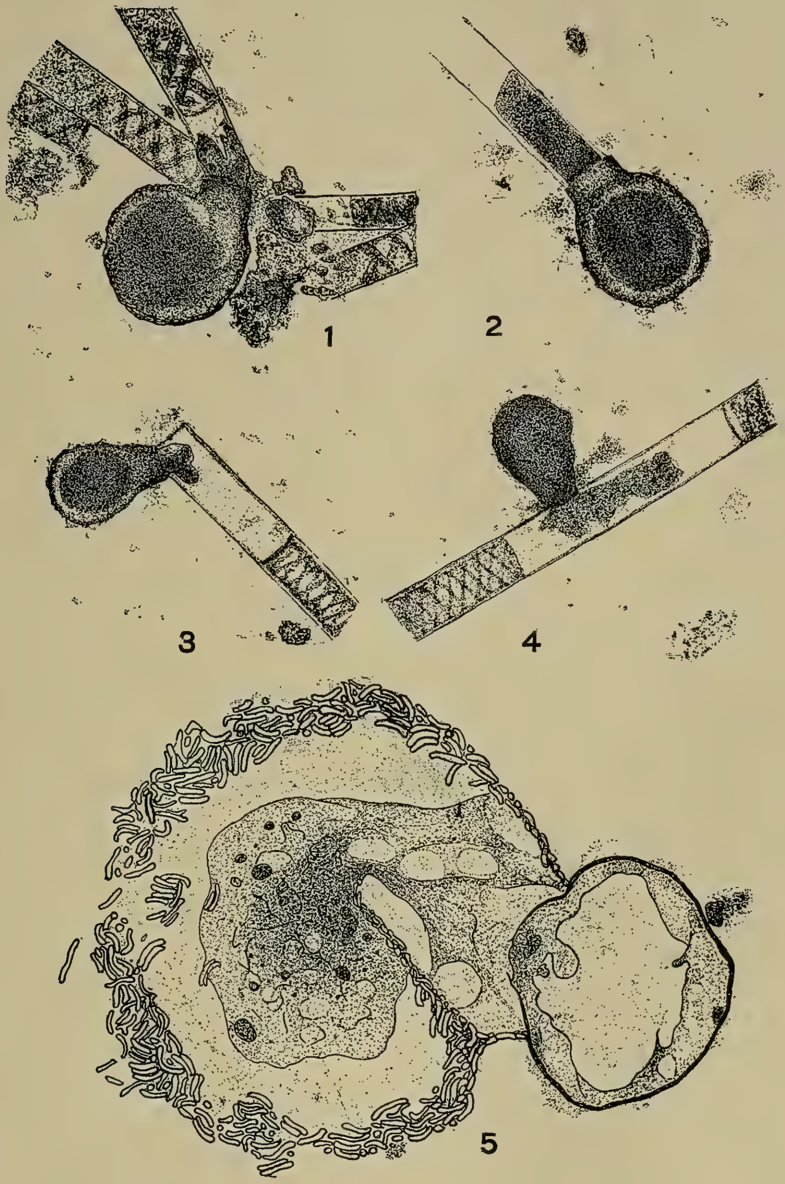


Plate I

Lesquereusia spiralis while feeding on *Spirogyra* (Fig. 5, Pl. I) show that the wall of the alga cell was considerably distorted from its cross-section circularity in the region covered by the mouth of the animal. Moreover the cell wall, which can be seen bent outward, is visibly thinner than the part which lies outside the mouth. This thinness here seems to show that some dissolving has taken place. Also, since *Lesquereusia* can ingest *Mougeotia* cells and contents and digest the cellulose, it is likely that the cellulose-digesting enzymes are present in the cytoplasm of the pseudopods. Calkins (1926) states that a number of rhizopods have been reported as digesting cellulose.

The openings made in the cell walls were always tears or rents, never the typical round holes left by *Vampyrella*. In the case of *Vampyrella,* where there is a sucking action, the filament gives a distinct snap when the softened cellulose gives way and an opening is made into the algal cell. This snap is not at all pronounced when a *Difflugia* or *Lesquereusia* opens up a cell. It seems that the pseudopods of these animals pull the softened cellulose until it tears open.

After having gained an entrance to the algal cell, the *Pontigulasia, Difflugia* or *Lesquereusia* devours the contents. The method by which the chloroplasts and cytoplasm are carried to the animal could not be ascertained with certainty. Possibly at this time there is some sucking action similar to that in *Vampyrella,* although this is doubtful. In one case this transportation of the algal protoplasm was seen going on in a pseudopod 60 microns long. This action appeared as a continuous streaming of the protoplasm carrying the chloroplasts and other material back to the shell mouth. Apparently this was due to cytoplasmic movement in pseudopods as explained by Mast (1931). Clearly here there could have been no sucking action since the process lasted continuously for fifteen minutes. No pumping, contracting, or other movement of the pseudopod, beside the steady streaming, was seen.

It was found impossible to observe the disposal of the food material in the living animal due to the opaqueness of the test. Sectioned animals, however, show that as the contents of a *Spirogyra* cell are being ingested large food vacuoles are formed in the anterior region. Later these large food vacuoles become smaller due to digestion or to their breaking up into smaller ones.

The color of these animals is governed to a great extent by the time elapsed since feeding. Just after devouring the contents of a *Spirogyra* cell their color is distinctly green. Later this green will change to brown and finally disappear. This color change is similar to that in *Vampyrella* (Lloyd, 1929) and is caused by the breaking down of the chlorophyll, leaving the carotin. The brown color will gradually disappear, leaving the animals a gray color and slightly transparent.

Some observations were made on two other species of *Difflugia, D. corona* and *D. urceolata.* It was found that these forms feed almost entirely on bacteria or unicellular algæ. In a few cases where filamentous algæ were used for food it was taken in through the mouth of the shell, both cell and contents being digested. *Centropyxis* was found to feed on *Spirogyra* in the same way, there being no attempt to remove the protoplast and leave the cell empty.

It was found that the large species of *Spirogyra* in good condition were immune from the attacks of any of these forms. *Lesquereusia spiralis* were seen to attempt an entry, attaching themselves in the usual manner but without any effect.

DISCUSSION

Because of the lack of information on the feeding habits of *Lesquereusia, Pontigulasia,* and *Difflugia,* the similarity with *Vampyrella* has been stressed. A great deal of work has been done on *Vampyrella* and its actions in attacking filamentous algæ are well known. For the most part the resemblance between thecamœbæ and *Vampyrella* is remarkable. The chief difference is the fact that there is apparently no sucking action by the Testacea in their feeding. The openings into the algal cells probably are made by the pulling of the pseudopods and the contents are removed by their cytoplasmic streaming. In *Vampyrella* the circular opening into the algal cell is caused by a dissolution of the cellulose wall followed by suction and it is thought that the contents are removed by suction also.

Very few of the *Difflugia* observed had what could be considered Zoöchlorellæ. In most cases the green colored bodies faded out on several days starvation, showing that they were food vacuoles. No *Lesquereusia spiralis* were found to have Zoöchlorellæ; their color depended entirely on the food ingested.

The feeding habit described seems to be fairly well distributed in the Testacea but is apparently restricted to the smaller species. It is probably a development of the normal feeding habits so that algæ too large to be taken through the mouth can be fed upon.

SUMMARY

1. *Lesquereusia spiralis, Pontigulasia vas, Difflugia constricta,* and *D. lobostoma* have been observed feeding on filamentous green algæ in a manner similar to that of *Vampyrella.*

2. The algal cells are entered by tearing or ripping the cell wall after it has been softened or partially digested.

3. Apparently there is no sucking action as for *Vampyrella;* the cell contents are removed by cytoplasmic streaming.

4. The entire process of opening the cell and removing the contents is completed in an hour.

5. Very little difference was noted in the feeding action of the four Testacea observed.

LITERATURE CITED

Calkins, G. N., 1926. Biology of the Protozoa. Lea and Febiger, Philadelphia and New York.

Cash, J., 1909. The British Freshwater Rhizopoda and Heliozoa. Vol. 2. The Ray Society, London.

Kudo, R., 1931. Handbook of Protozoölogy. C. C. Thomas, Springfield, Illinois and Baltimore, Maryland.

Leidy, J., 1879. Fresh-water Rhizopods of North America. U. S. Geological Survey of the Territories. Government Printing Office, Washington, D. C.

Lloyd, F. E., 1929. The Behavior of Vampyrella lateritia with Special Reference to the Work of Professor Chr. Gobi. *Arch. Protist.,* **67**: 219.

Mast, S. C., 1931. Movement and Response in Difflugia. *Biol. Bull.,* **61**: 223.

Penard, E., 1902. Faune Rhizopodique du Bassin du Lemain. W. Kundig et Fils, Geneve.

THE EFFECTS OF ANTUITRIN–S ON THE MALE GENITAL ORGANS OF THE LIZARD (*EUMECES LATICEPS*)[1] DURING SEASONAL ATROPHY[2]

CLARENCE D. TURNER

(*From the Department of Zoölogy, the University of Georgia*)

INTRODUCTION

The present investigation was undertaken to determine the effects of anterior-pituitary-like substances from pregnancy urine upon an animal in which there is marked seasonal periodicity in reproductive activity. Although there are many accounts dealing with the physiological and gross effects of these extracts, the majority of them have been confined to mammals. In rats and mice, prespermatogenesis begins at an early period, and there is little subsequent variation in the activity of the gonads and accessories. Results with these animals are often complicated by the possibility of spontaneous maturity before completion of the experiment, or rendered obscure by rapid spermatogenesis and elaboration of secretory products. It was thought that more conclusive results might be obtained by studying the histological and cytological effects of these extracts upon the genital complex of adult *Eumeces* during the period of low sexuality.

Pregnancy urine extracts, injected into normal animals, are reported to increase the weight of the testes, cause at least temporary hypertrophy of the interstitial tissue of the testis, and induce precocious development of the accessory organs. Smith and Leonard (1934), however, observed in hypophysectomized rats a regression of the accessories and interstitial tissue by the thirtieth day of treatment with pregnancy urine. In some of the unoperated animals they observed regression of the accessories after prolonged (62-day) treatment.

---

[1] The writer is indebted to Professor E. H. Taylor, Department of Zoölogy, University of Kansas, for verifying the identity of this species. Acknowledgments are due to Parke Davis and Company for their courtesy in supplying a portion of the Antuitrin–S used in these experiments, and also to the Department of Zoölogy, University of Missouri, for the microphotographs. The author is further indebted to Professor G. H. Boyd, University of Georgia, for many helpful criticisms, and to Mr. William Buchanan and Mr. Oliver Allen, students at the University of Georgia, who have given much technical assistance during the course of these experiments.

[2] The present investigation was begun during January, 1934 and abstracted in the Proceedings of the Georgia Academy of Science on March 22, 1935. At present, these observations are being extended with other endocrine extracts and upon both sexes subsequent to various glandular operations.

Findings with reference to the effects of pregnancy urine upon the seminiferous tubules have not been consistent. Engle (1932), using pregnancy urine or pyridine extract of the anterior pituitary, observed that in immature rats there was no acceleration of mitoses in the germinal epithelium, and that in some cases there was an extensive degeneration of the tubules. This cellular degeneration he did not regard as due to differences in amount of the extract administered or to the length of treatment. The extract (gravidin) used by De Jongh (1930) and Kraus (1930, 1931) was claimed to have no effect on the germinal epithelium of rodents. Smith and Leonard (1934) reported that pregnancy urine extracts retarded testicular atrophy which normally follows pituitary ablation in the rat, and that in hypophysectomized adults spermatogenesis was maintained when treatment was begun immediately following the operation. However, they were not able to induce mature spermatozoa in immature hypophysectomized animals by pregnancy urine injections, although the tubules were maintained. Their treatment did not hasten sexual maturity in unoperated males, but in no case did they observe injury to the seminiferous epithelium. Herlant (1931) reported no pronounced effect of pregnancy urine upon the seminiferous tubules of hibernating hedge hogs. Wells (1934) was able to induce the formation of spermatozoa in the ground squirrel during the period of low sexuality by the administration of untreated pregnancy urine, Antuitrin-S, and fresh rat-pituitary implants. Schockaert (1931) found that the injection of anterior pituitary substances caused spermatogenesis in immature ducks. Brosius (1935) and others have concluded from clinical observations that anterior-pituitary-like substances stimulate spermatogenesis and hasten descent of the human testicle.

Thus some workers have found an inhibition in the development of the seminiferous tubules, or even injury to the epithelial cells; while others have reported increased tubule development and different degrees of spermatogenesis. This lack of uniformity appears to be due to physiological differences in the experimental animals, degree of purity of the extract, and the amount of treatment. Generic differences may also be important factors.

MATERIAL AND METHODS

The observations here reported are based on a study of 154 male skinks obtained through the Florida Reptile Institute, Silver Springs, Florida. In order to determine the seasonal periodicity, testes and accessory ducts were fixed and sectioned during each month of the year. During the season of lowest sexuality (October, November, and December) 0.2 to 0.4 cc. (20 and 40 rat units) daily injections of Antuitrin-S were administered intramuscularly to adult males, and these were autopsied at intervals of 4, 9, 10, 20, and 30 days. Material was fixed at these intervals for histological and cytological studies. Less extensive experiments were undertaken with untreated pregnancy urine.[3]

Bouin's picro-formol followed by Delafield's hematoxylin, or Bullard's modification [4] of Ehrlich's hematoxylin, and eosin were used most extensively for histological purposes. When cytological observations were to be made, the animal was killed by decapitation or by a blow on the head, and small pieces of the tissue quickly dissected and dropped into the fixative. Ludford's modification of the Mann-Kopsch method was used most extensively for studies on the Golgi apparatus, although comparisons were made with tissues fixed according to the Kolatchev method as modified by Nassonov. After the latter initial fixation, the material was osmicated for 5, 10 and 20 days at 35° C. Oil of turpentine or 0.01 per cent aqueous potassium permanganate were used as bleaching agents. Material fixed in Champy's or Helly's fluid and stained by the anilin-fuchsin-toluidin-aurantia method of Kull was used for the study of chondriosomes. The latter fixative gave the most satisfactory results.

Observations

Weight and Histology of the Testes

The Testes During Atrophy.—There is marked seasonal variation in the male genital organs of *Eumeces.* The testes and accessory ducts atrophy during the autumn and early winter and remain practically quiescent until early spring and summer. Mature spermatozoa normally appear during early April, and breeding begins in May.

During involution the seminiferous tubules are comparatively small as a result of atrophy and aplasia of the germinal epithelial cells (Fig. 1). The " weight of " testes from 55 control animals autopsied during October, November and December averaged 53.22 mgm. At this period the tubules range in size from 115 to 180 μ in diameter, with an average of 160 μ. During the breeding season the weight of the testes averages approximately 160 mgm. and the diameters of the tubules vary from 200 to 360 μ, the average being 330 μ. At seasonal involution the tubules appear collapsed and the lumina are inconspicuous. The latter are partly closed by a loose syncytium, the interspaces of which often contain degenerating cells. No spermatids or spermatozoa are present and mitotic divisions are seldom encountered (Fig. 3). Many degenerating epithelial cells with pycnotic nuclei are present throughout the testes at this period. Although we have observed no evidence of hyperplasia of the Sertoli cells, they are very conspicuous features of the quiescent testis and have apparently been brought into greater prominence through the atrophy and aplasia of the spermatogonia and sperma-

---

[3] Pregnancy urine was made available by Dr. H. B. Harris, Athens, Georgia.

[4] See Laboratory Methods of the U. S. Army, Manual No. 6, p. 69.

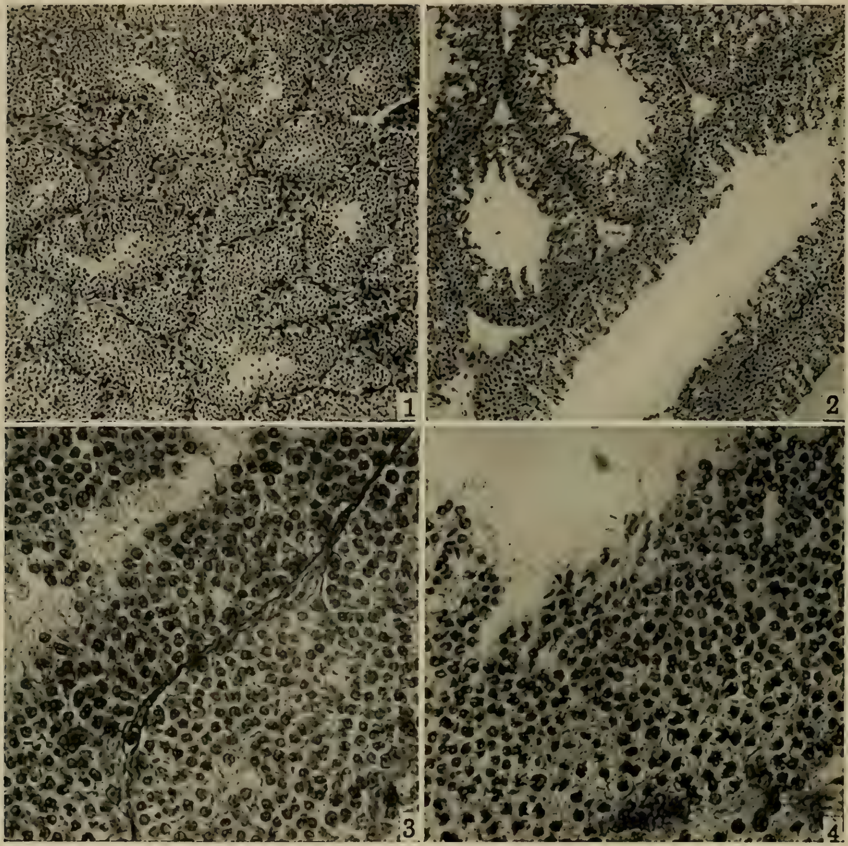


FIG. 1. An involuted testis from a control. Bouin-hematoxylin. × 55.

FIG. 2. An involuted testis subsequent to 30-day treatment with Antuitrin–S (total 600 r. u.). Bouin-hematoxylin. × 55.

FIG. 3. Higher magnification of a portion of Fig. 1. Note absence of mitotic figures. Bouin-hematoxylin. × 390.

FIG. 4. Higher magnification of a portion from Fig. 2. Mitoses are numerous and a few mature spermatozoa are present in the lumen. Bouin-hematoxylin. × 390.

tocytes. The Sertoli cells are confined to the periphery of the tubules and their longer axes are usually parallel to the basement membrane.

The Testes Following Antuitrin-S Administration.—The administration of Antuitrin-S causes the testes to increase greatly in size and weight. At autopsy these organs appear much more turgid and are in marked contrast to the collapsed and more flabby testes of the controls. In 25 adults that received 30 daily injections of Antuitrin-S, the weight of the testes increased 136.70 per cent over a similar number of controls (Table I, Experiment 5). As can be seen by comparing the experiments summarized in Table I, the growth of the testes during atrophy is proportional to the length of treatment and the amount of extract administered. The effect of the extract upon the testes is much less pronounced during the onset of sexual activity (compare Experiments 3 and 7).

Two sexually inactive animals were given 20 rat units of Antuitrin-S daily for 30 days, laparotomized at this time, and autopsied 20 days after discontinuing the treatment. These showed enlarged testes and accessory ducts at laparotomy, but at autopsy the organs had involuted until they approximated the condition normally found in *Eumeces* during seasonal atrophy.

Histological preparations from treated animals (Experiment 5) show marked enlargement of the seminiferous tubules (Figs. 2 and 4). Their diameters range from 205 to 320 μ, with an average of 257 μ. The lumina of the tubules are large and open, thus giving the testis a porous appearance when examined macroscopically. The anastomosing cells which are usually present in the lumina of control testes have disappeared after treatment for 30 days. Degenerating cells in the germinal epithelium are infrequently observed after treatment for 10 days, and are practically absent after 30 days. After the administration of 600 r. u. over a period of 30 days (Experiment 5) early stages of spermatogenesis are observed throughout the testis, and a few mature spermatozoa are observed in the lumina (Fig. 4). Twenty rat units of Antuitrin-S administered twice daily for a period of 20 days (Experiment 6) initiates rapid spermatogenesis. The seminiferous tubules become crowded with mature spermatozoa which accounts for the greater weight of this organ. The number of Sertoli cells appears not to be altered, although they are less conspicuous than in controls due to the crowded condition resulting from the proliferative phase of spermatogenesis. Most of the Sertoli cells of the treated testis are elongated and extend vertically from the basement membrane toward the lumen. Although actual measurements have not been made, the amount of interstitial material appears to be slightly increased. This seems to be due

TABLE I

The Effect of Antuitrin-S on the Weight of the Testes and Epididymides in Eumeces laticeps[5]

Experiment	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7
Date of autopsy	Oct. 8, 1934	Oct. 8, 1934	Nov. 11, 1934	Dec. 18, 1934	Dec. 18, 1934	Jan. 15, 1935	Apr. 20, 1935
Average body weight of experimental animals, *gm.*	30.01	30.90	29.63	31.97	28.90	27.93	31.44
Number of experimental animals	6	6	12	6	25	5	10
Number of days injected	4	4	10	20	30	20	10
Rat units per day	20	40*	20	20	20	40*	20
Average body weight of control animals, *gm.*	30.00	29.62	30.41	30.17	29.02	31.08	30.49
Number of controls	6	6	12	6	25	3	10
Average weight of experimental testes, *mgm.*	57.41	65.23	73.01	92.50	123.87	152.61	162.35
Average weight of control testes, *mgm.*	52.22	53.64	52.34	55.57	52.33	61.32	133.73
Increase in weight of testes, *per cent*	9.94	21.60	39.49	66.45	136.70	148.87	21.40
Average weight of experimental epididymis and vas deferens, *mgm.*	10.00	10.03	12.61	14.70	19.02	26.98	25.90
Average weight of control epididymis and vas deferens, *mgm.*	8.01	6.30	7.26	8.22	8.17	8.93	21.00
Increase in weight of epididymis and vas deferens, *per cent*	24.84	59.20	73.70	78.83	132.80	202.12	23.33

* Twenty rat units injected twice daily.

---

[5] It should be emphasized that these results may hold only for this particular amount and duration of treatment.

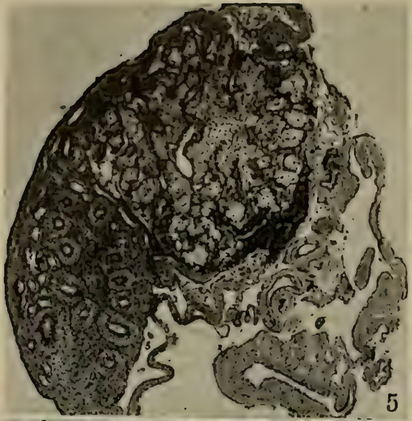
mostly to an increase in the size of the cells, although there remains the possibility that there is some increase in numbers through the differentiation of mesenchymal cells which are indistinguishable from fibroblasts. No regression of the interstitial cells has been observed in *Eumeces* as described in normal and hypophysectomized male rats by Bourg (1931) and Smith and Leonard (1934) following pregnancy urine treatment for 30 days. It is therefore possible by the administration of pregnancy urine to transform an involuting testis into one that simulates the condition normally prevailing during the height of the breeding season.

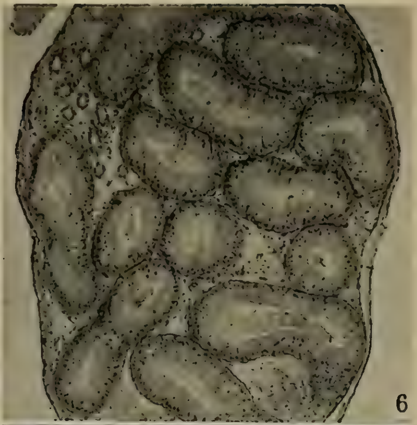
Untreated urine from pregnant women has the same influence on the testis as Antuitrin-S, although the effects are not so pronounced and the large amounts that must be injected are more toxic to the animals. The testis and epididymis are caused to enlarge slightly, early stages of spermatogenesis are induced, but it has not been possible to administer sufficient amounts to cause the formation of spermatids and mature spermatozoa.

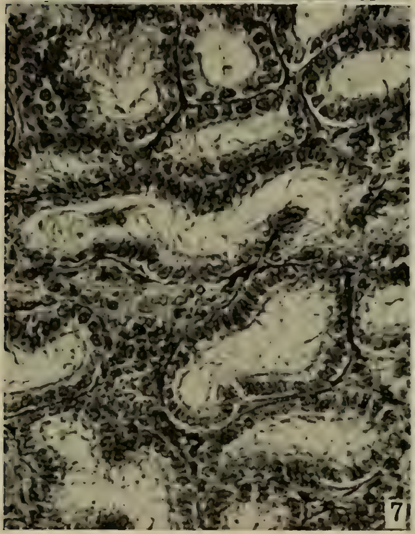
Weight and Histology of the Epididymis and Vas Deferens

The Epididymis and Vas Deferens During Atrophy.—Since there is no line of demarcation that may be employed upon gross examination to distinguish accurately the epididymis and vas deferens, these two structures were considered together. When these were to be weighed, the adrenal gland was dissected free from the epididymis and the vas deferens severed as closely as possible to the cloaca. During atrophy these combined structures from 55 animals averaged 7.59 mgm. The epididymis is surrounded by a compact connective tissue layer which is continuous with the adrenal capsule (Fig. 5). When corresponding levels of the epididymides in control and experimental animals were desired, sections were selected that passed through the anterior or posterior ends of the adrenal glands. The tubules of the involuted epididymis vary from 18 to 42 μ in diameter. They are surrounded by a thin layer of smooth muscle cells, and the connective tissue between them is very compact and comparatively non-vascular. The epithelium consists of a single layer of low cuboidal cells which average 11 μ in height (Figs. 5 and 7). Few if any secretory materials can be observed in the cells, or in the lumina. No mitotic figures have been observed in any of the epithelial cells of the epididymis during seasonal quiescence.

The vas deferens is a tortuous tube extending from the epididymis to the cloaca. It is lined by a pseudostratified columnar epithelium which is not appreciably secretory. Two or three layers of nuclei are







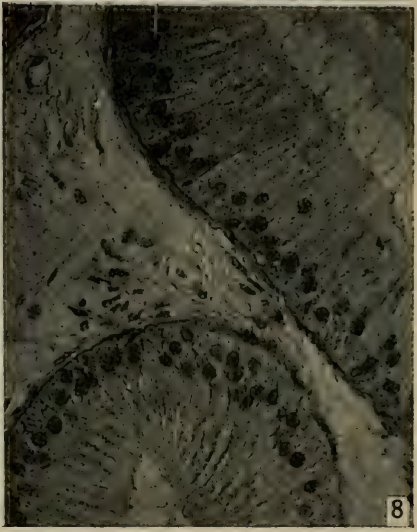


Fig. 5. Cross-section of the epididymis (lower left) and adrenal gland of a control during the period of low sexuality. Bouin-hematoxylin. × 55.

Fig. 6. Cross-section of the epididymis from an animal during atrophy that received treatment for 30 days with Antuitrin–S (total 600 r. u.). Section passes through approximately same level as Fig. 5. Bouin-hematoxylin. × 55.

Fig. 7. Higher magnification of a portion of the involuted epididymis. Bouin-hematoxylin. × 390.

Fig. 8. Section of an involuted epididymis following treatment with Antuitrin–S for 30 days (total 600 r. u.). Higher magnification of a portion from Fig. 6. × 390.

present in the epithelial layer, and cilia are absent. External to the basement membrane are comparatively thick longitudinal and circular layers of smooth muscle. A compact layer of fibrous connective tissue covers the tube peripherally.

The Epididymis and Vas Deferens Following Antuitrin-S Administration.—Following the administration of Antuitrin-S for 30 days (Table I, Experiment 5), the epididymis and vas deferens increased 132.80 per cent over a similar number of controls. In this experiment a few mature spermatozoa were present in the seminiferous tubules, but practically none were to be found in the lumina of the epididymis. The increase in weight of the epididymis is not due to the accumulation of spermatozoa, but to the hypertrophy and hyperplasia of the secretory epithelium, as well as to the vascularization of the intertubular connective tissue, and to secretory materials in the lumina (Figs. 6 and 8). Most of the secretion present in the lumina of the epididymis at this time has been produced by the epithelial cells of this organ, although there is the possibility that a portion of it has been received from the testis. After treatment as in Experiment 5, the tubules of the epididymides range from 176 to 220 μ in diameter. The average was found to be 186 μ. The height of the epithelial cells varies from 40 to 68 μ, the average being 56 μ. Thus the height of the epithelium is at least five times greater than in controls. Mitoses are frequent in this layer. These elongated cells lose connection with the basement membrane, become rounded, and undergo mitoses near the luminal border of the epithelium. Therefore, there is not only hypertrophy but hyperplasia of these cells. If cilia are present during the period of accelerated secretion they are obscured by the secretion. The distal ends of the cells are packed with secretory granules, and secretion is contained in the lumina. The intertubular connective tissue is less dense and has obviously increased in amount (Figs. 7 and 8). The muscle layer appears thinner than in controls, this apparently resulting from enlargement of the tubules without a corresponding increase in the number of muscle cells. Among the 25 treated animals in Experiment 5, practically no individual variation was observed in the response of the epididymides to pregnancy urine treatment.

The control epididymides during April (Experiment 7) are much heavier than during October, November and December. This increase in weight is due to the onset of secretory activity in the organ itself, preparatory to the breeding season, and to the accumulation of testicular products. In Experiments 6 and 7 the administration of Antuitrin-S hastened the passage of spermatozoa into the epididymides, and this partially accounts for the greater weight of these organs than in Experiment 5. In the latter experiment, the pregnancy urine treatment accelerated secretory activity in the epididymis, but practically no spermatozoa were liberated from the testis.

Treatment with Antuitrin-S induced mitoses in the epithelial cells of the vas deferens. The epithelium thickens until it is composed of 4 or 5 layers of cells. Other than this, there is no pronounced effect on the vas deferens.

Cytological Changes in the Epididymis

Cytological observations on the secretory epithelium during atrophy and subsequent to Antuitrin-S administrations were not undertaken to determine the rôle of the Golgi bodies and chondriosomes in the elaboration of secretions, but to determine the more apparent cellular changes as indicators of the effects of injecting the gonadotropic extract. The detailed behavior of the cytoplasmic inclusions during synthesis of the secretory products belongs more to the province of pure cytology than to the main interests of the present investigation. We have, therefore, used these changes as criteria for determining the minute effects of the endocrines concerned, but have refrained from drawing conclusions as to the functional significance of the inclusions except as they may be regarded as general indices of cellular physiology.

The Epididymis During Seasonal Atrophy.—The secretory epithelium of the tubules is composed of a single layer of cuboidal cells which average 11 μ in height (Fig. 11). The nucleus is elliptical and the cell barely exceeds it in width. It contains from 1 to 3 heavily staining nucleoli and smaller chromatin granules distributed in the nuclear substance. Nucleolar extrusions into the cytoplasm as described by Ludford (1925) in the epithelium of the epididymis of the mouse have not been observed in *Eumeces*. The supranuclear portion of the cell contains small secretory granules that are best demonstrated by the techniques employed for chondriosomes. A few rod-shaped chondriosomes are present near the nucleus (Fig. 11). If spherical chondriosomes are present in addition to rods, they are not discernible from the fuchsinophilic granules. We regard these granules as secretory since they appear to be liberated from the cells and are identical to substances found in the lumina. The epithelial cells during atrophy contain from 1 to 3 neutral fat globules in the supranuclear region. In a few of these cells a small Golgi apparatus is present distal to the nucleus, but in most cases no Golgi reticulum is discernible. There are, however, numerous small osmiophilic granulations that are not bleached by turpentine or potassium permanganate any more rapidly than the Golgi bodies of other cells.

The largest granules often appear grouped close to the nuclear membrane, in the supranuclear area, and are frequently surrounded by an osmiophilic zone of cytoplasm. This zone bleaches slowly, but in advance of the granules here referred to. It seems likely that when the cells are not highly secretory, as during seasonal atrophy, the Golgi apparatus is represented only by these osmiophilic granules, and that it is reconstructed from them concurrently with accelerated secretory activity (Figs. 9 and 10). A similar behavior of the Golgi apparatus has been described by Brambell (1925) in the epithelial cells of the oviduct of the fowl during retarded and accelerated secretion.

The Epididymis Following Antuitrin-S Administration.—There is a gradual increase in the number of epithelial cells through mitotic divisions. No evidence of amitosis has been observed among these cells as Ludford (1925) described in the mouse epididymis. A few basal cells are present in the epithelium before and after treatment, but these are not increased in numbers following the injections. Neither have these been observed in mitotic division at any period. There is no evidence indicating that the cuboidal cells of the involuted epithelium are lost and subsequently replaced through differentiation of basal cells. The columnar cells are frequently observed undergoing mitoses following Antuitrin-S injections. These dividing cells are clearly the columnar cells of the epithelium, as is indicated by the fact that they may advance as far as the metaphase before losing connection with the basement membrane or withdrawing from the luminal margin of the epithelium. In animals that received treatment for 30 days (Experiment 5), the cells average 56 μ in height. This elongation is apparently due to

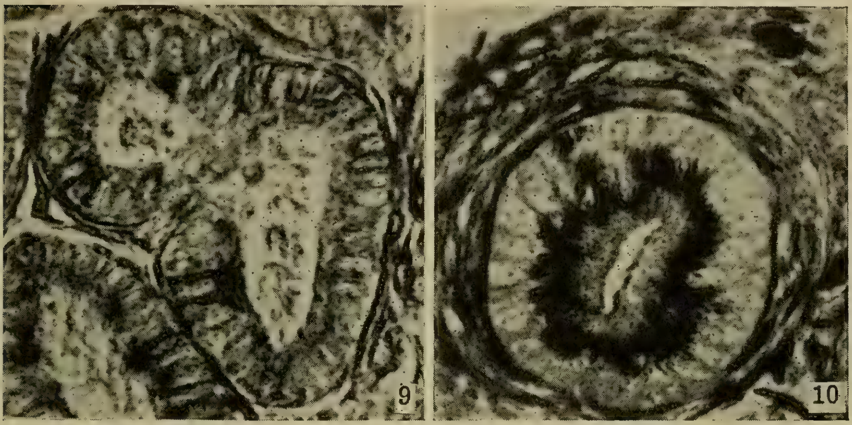
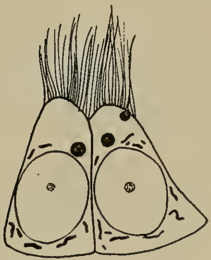


FIG. 9. Cross-section of tubules from the epididymis of a control during lowest sexuality. Mann-Kopsch and unstained. Bleached in oil of turpentine for 30 days. × 410.

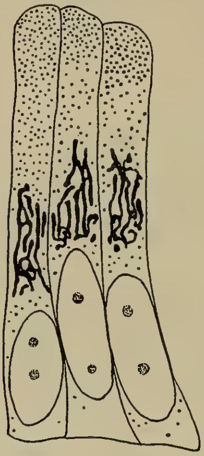
FIG. 10. Cross-section of tubule from an involuted epididymis following treatment with Antuitrin–S for 30 days (total 600 r. u.). The epithelial cells are much elongated and Golgi bodies are conspicuous in the supranuclear region. Mann-Kopsch and unstained. Bleached in oil of turpentine for 30 days. × 410.

crowding, and also to an increase in cytoplasmic volume. There is very little increase in the volume of nuclear material.

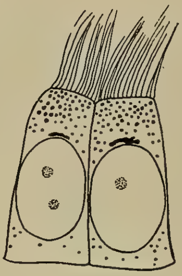
The distal ends of the cells contain many small fuchsinophilic inclusions. Some of these secretory granules are osmiophilic, but bleach in advance of the Golgi reticulum. Neutral fat globules, as present in these cells during atrophy, have disappeared entirely. These greatly elongated cells contain large Golgi bodies composed of osmiophilic strands interwoven into complicated forms (Figs. 12 and 13). The Golgi elements occur in the supranuclear region slightly distal to the nucleus. This hypertrophy of the Golgi apparatus is comparable to the results obtained by Moore et al. (1930, *a*, *b*), who studied the effects of the male hormone on the cytology of the prostate and seminal vesicles of castrate rats.



11



12



13

FIG. 11. Involuted secretory epithelial cells from a control epididymis, showing rod-shaped chondriosomes and several neutral fat globules. Champy-Kull. × 1463.

FIG. 12. Involuted epithelial cells of the epididymis subsequent to Antuitrin–S injections (total 200 r. u.) for 10 days. The Golgi apparatus is enlarged and has moved slightly away from the nucleus. The smaller inclusions are fuchsinophilic granules. Mann-Kopsch followed by Altmann's acid fuchsin. × 1463.

FIG. 13. Involuted secretory epithelial cells of epididymis after injections with Antuitrin–S (total 40 r. u.) for 2 days. Spherical fuchsinophilic granules are most abundant in the luminal region of the cells. Definite Golgi bodies are apparent immediately distal to the nucleus. Mann-Kopsch followed by Altmann's acid fuchsin. × 1463.

Chondriosomes in the form of rods have become abundant near the nucleus, but the area occupied by the Golgi reticulum is relatively free from them. There is here a very obvious hyperplasia of the chondriosomes over similar cells from untreated animals during atrophy. This further confirms the observations of Nassonov (1924) and Ludford (1925), both of whom reported an increase in the number of chondriosomes at the onset of secretory activity in the untreated mammalian epididymis.

Many of the epithelial cells at this period have developed knob-like or finger-shaped cytoplasmic processes from their luminal surfaces. These cytoplasmic blebs are filled with small secretion granules and are presumably freed into the lumen of the tubule.

Discussion

In our earlier experiments on *Eumeces,* treatment with Antuitrin-S was continued for only a short period (5 to 10 days) and seemed to give results that were harmonious with those on mammals by Steinach and Kun (1928), Borst and Gostimirović (1930), Boeters (1930), Bourg (1931), and Smith and Leonard (1934). All of these investigators reported acceleration of spermatogenetic activity, without spermatids or spermatozoa, following treatment with gonadotropic substances. The heavier treatments that we administered induced the formation of mature spermatozoa and support the claims of Voss and Loewe (1928), Belawenetz (1930), and Wells (1934). All of these found that gonad-stimulating extracts from the anterior pituitary or urine of pregnancy induced the formation of mature spermatozoa in mammals. Such deviations in the degree of spermatogenesis are apparently due to variations in the amount and duration of treatment, and indicate the importance of employing assayed preparations of known potency in experimental and clinical procedures.

The data presented, showing that Antuitrin-S stimulates spermatogenesis, are in accord with the known clinical value of this extract in the treatment of aspermia and cryptorchidism. Observations on *Eumeces* that have not been presented indicate that the accessory organs are indirectly stimulated through the testis. Following bilateral castration during the height of the breeding season, the epididymis undergoes involution until it approximates or surpasses the atrophic condition at the period of lowest sexuality. In the absence of the testes, the epididymides are not maintained by Antuitrin-S injections. Wells (1934) reported the failure of gonadotropic substances to stimulate the accessories of castrate ground squirrels.

Antuitrin-S, when administered to female lizards, has not been observed to exert any significant influence on the weight of the ovaries, although further studies are in progress. Neither have we been successful in inducing ovulation by use of this extract as was accomplished by Ogilvie (1933) in amphibians. However, when administered during seasonal involution, very pronounced changes are produced in the oviducts. These organs increase in weight due to glandular development and elongation of the epithelial cells. During atrophy the oviduct has comparatively thin walls and the convolutions are not pronounced. By injection of this agent during seasonal quiescence this organ can be caused to simulate the condition that normally prevails during the period of highest sexuality.

Experiments on *Eumeces* to determine the effects of Theelin on the genital system of both sexes are in progress. The daily administration of 20 rat units of Theelin (in oil) to adult males for a period of 15 days causes a decrease in the weight of the testis, and an increase in the weight of the epididymis. Using 12 experimental animals and a similar number of controls, taken during the breeding season, the testes from injected animals were 33.34 per cent inferior in weight to their respective controls. The epididymides subsequent to this treatment with Theelin increased 40.08 per cent in weight. Histological preparations did not reveal significant alteration in the degree of spermatogenesis. However, many spermatids and other immature cells were observed free in the lumina of the seminiferous tubules. These immature elements were also present in the epididymides. The secretory epithelium of the epididymis at this season does not appear to be altered to any appreciable degree subsequent to 15-day treatment with Theelin. The rapid proliferation of cells from the germinal epithelium and the passage into the epididymis offers only a partial explanation of the decrease in weight of the testis, and the increase in weight of the epididymis. The fact that the amount of material gained by the epididymis is not equivalent to that lost from the testis indicates that additional material is resorbed from one or both of these organs. Although the proliferation of immature cells from the germinal epithelium following injections of estrogenic material is apparently to be regarded as degenerative, there is an abundance of mature sperm in both the testis and epididymis. Notwithstanding the lack of actual data on fertility in this species, one may postulate from histological studies of control and treated testes that the fertility would not be wholly destroyed subsequent to this amount of treatment. While prolonged experiments with Theelin are necessary, these results on *Eumeces* are harmonious with those of Wade and Doisy (1935) and others who subjected male mammals to the influence of the female sex hormone and observed slight impairment of the genital system.

SUMMARY AND CONCLUSIONS

1. The daily administration of 20 rat units of Antuitrin-S elicits an increase in the weight of the involuting testis, and epididymis and vas

deferens. In 25 animals receiving this treatment for 30 days, the testes increased 136.70 per cent and the epididymis and vas deferens (collectively) 132.80 per cent over a similar number of controls. This treatment induced the formation of a few mature spermatozoa, but practically none of them were liberated into the epididymides. Enlargement of the epididymis is here due to the initiation of secretory activity in the epithelial cells of this structure.

2. The administration during seasonal atrophy of 20 rat units of Antuitrin-S twice daily for a period of 20 days increased the weight of the testis 148.87 per cent, and the epididymis 202.12 per cent. Many mature spermatozoa were present in both the testis and epididymis. The increased weight of the epididymis over that observed in animals subjected to lighter treatment is due mostly to the accumulation of spermatozoa in this organ.

3. During the period of high sexuality the response of the testis and epididymis to treatment with Antuitrin-S is less pronounced than during seasonal quiescence (Experiments 5 and 7).

4. There are marked histological and cytological alterations in the involuted epididymis following injections of anterior-pituitary-like substances. The intertubular connective tissue increases in amount, becomes highly vascularized, and the cells of the secretory epithelium undergo hypertrophy and hyperplasia. An extensive Golgi apparatus is induced in the epithelial cells. Likewise there is an increase in the number of chondriosomes, and the formation of secretory blebs that are freed into the lumina. These observations indicate accelerated secretory activity as the result of Antuitrin-S injections. The gonadotropic extract apparently exerts its influence on the epididymis through the testis since it is not maintained by the extract when administered to castrates.

5. The cuboidal cells lining the tubules of the involuting epididymis average 11 μ in height. Following the injection of 600 rat units of Antuitrin-S over a period of 30 days, these cells average 56 μ in height.

6. The vas deferens shows little response to Antuitrin-S treatment, although there is a thickening of its walls due to mitotic multiplication. This simulates the condition normally found in untreated animals during the breeding season.

7. At seasonal involution, anterior-pituitary-like substances induce enlargement of the oviducts due to glandular development and to stimulation of the epithelium. No gross effects of this extract have been observed on the ovary.

8. The administration of 20 rat units of Theelin (in oil) for 15 days to adult males collected during the breeding season increases the weight of the epididymis and decreases the weight of the testis.

LITERATURE CITED

Belawenetz, S., 1930. Über die Wirkung des Pituitrins auf das Wachstum und die Hoden der weissen Ratten. *Virchow's Arch.*, **274**: 585.

Boeters, H., 1930. Prolanversuche an jungen männlichen Ratten. *Deut. med. Woch.*, **56**: 1382.

Borst, M., and D. Gostimirović, 1930. Weitere Ergebnisse über die Beziehungen zwischen dem Geschlechtshormon des Hypophysenvorderlappens und der männlichen Keimdrüse. *Münch. Med. Woch.*, **77**: 1536.

Bourg, R., 1931. Recherches sur l'histophysiologie de l'ovaire, du testicule et des tractus génitaux du Rat et de la Souris. *Arch. d. Biol.*, **41**: 245.

Brambell, F. W. R., 1925. The Part Played by the Golgi Apparatus in Secretion, and its Subsequent Reformation in the Cells of the Oviducal Glands of the Fowl. *Jour. Roy. Micros. Soc.*, March, p. 17.

Brosius, W. L., 1935. Clinical Observations on the Effects of A P L (Antuitrin-S) on the Testicle. *Endocrinology*, **19**: 69.

De Jongh, S. E., 1930. L'hormone du lobe antérieur de l'hypophyse et les organes génitaux mâles. *Arch. Néerl. Physiol.*, **15**: 470.

Engle, E. T., 1932. The Action of Extracts of Anterior Pituitary and of Pregnancy Urine on the Testes of Immature Rats and Monkeys. *Endocrinology*, **16**: 506.

Herlant, M., 1931. Influence des injections d'urine de femme enceinte sur le tractus génital male du hérisson hibernant. *Compt. rend. Soc. de Biol.*, **106**: 1262.

Kraus, E. J., 1930. Wie Wirkung des Prolan (Ascheim-Zondek) auf die männlichen Geschlechtsorgane auf Grund von Versuchen an Maus und Ratte. *Kl. Woch.*, **9**: 1493.

Kraus, E. J., 1931. Zur Wirkungsweise des Prolans. *Arch. Gynäk.*, **145**: 524.

Ludford, Reginald James, 1925. Cell Organs during Secretion in the Epididymis. *Proc. Roy. Soc. London*, Series B, **98**: 354.

Moore, C. R., D. Price, and T. F. Gallagher, 1930a. Rat-prostate Cytology as a Testis-hormone Indicator and the Prevention of Castration Changes by Testis-extract Injections. *Am. Jour. Anat.*, **45**: 71.

Moore, C. R., W. Hughes, and T. F. Gallagher, 1930b. Rat Seminal-vesicle Cytology as a Testis-hormone Indicator and the Prevention of Castration Changes by Testis-extract Injection. *Am. Jour. Anat.*, **45**: 109.

Nassonov, D., 1924. Das Golgische Binnennetz und seine Beziehungen zu der Sekretion. *Arch. f. mikr. Anat.*, **100**: 433.

Ogilvie, A. E., 1933. Induced Ovulation in Amphibians by Injection of Antuitrin–S. *Proc. Soc. Exper. Biol. and Med.*, **30**: 752.

Schockaert, J. A., 1931. Response of the Male Genital System of the Immature Domestic Duck to Injections of Anterior-pituitary Substances. *Anat. Rec.*, **50**: 381.

Smith, P. E., and Samuel L. Leonard, 1934. Responses of the Reproductive System of Hypophysectomized and Normal Rats to Injections of Pregnancy-urine Extracts. *Anat. Rec.*, **58**: 145.

Steinach, E., and H. Kun, 1928. Die entwicklungsmechanische Bedeutung der Hypophysis als Aktivator der Keimdrüseninkretion. *Med. Klin.*, **24**: 524.

Voss, H. E., and S. Loewe, 1928. Geschlechtsprägende Wirkungen der Hypophysenvorderlappens am Männchen. *Pflüger's Arch.*, **218**: 604.

Wade, N. J., and E. A. Doisy, 1935. The Prolonged Administration of Theelin and Theelol to Male and Female Rats and its Bearing on Reproduction. *Endocrinology*, **19**: 77.

Wells, L. J., 1934. Sexual Periodicity in the Male Ground Squirrel (Citellus tridecemlineatus). *Anat. Rec.* **60** (supplement to No. 4) : 53.

THE ORIGIN AND NATURE OF THE FERTILIZATION MEMBRANE IN VARIOUS MARINE OVA

H. Y. CHASE[1]

(*From the Hopkins Marine Station, Stanford University*)

INTRODUCTION

A widely debated question in the literature is whether the membrane which develops after fertilization pre-exists on the unfertilized egg, merely being lifted and altered in its properties, or whether it actually forms *de novo* as a product of the cortical reaction. The appearance of the fertilization membrane was described first by Fol (1877, 1879), who thought that the membrane pre-existed on the unfertilized egg of the starfish as an enveloping cover. Many observers since have reported the lifting of a membrane from the surface of the egg following activation. O. and R. Hertwig (1887) found that membranes formed on fertilized fragments of ripe sea urchin eggs and concluded from this that membranes pre-existed. Herbst (1893) showed by crushing unfertilized sea urchin eggs and pressing out the contents that a delicate film-like membrane was left behind. Kite (1912) found a membrane on the unfertilized sea urchin egg by the microdissection method; Glaser (1914) for the sea urchin and starfish, F. R. Lillie (1919) for *Nereis,* Chambers (1921) for the starfish, sea urchin, and sand dollar, and A. R. Moore (1930) for the sand dollar, have described a membrane on the unfertilized egg. Hyman (1923) found a definite membrane of considerably greater consistency than the cytoplasm on the mature unfertilized eggs of the sea urchin and starfish. On the other hand, Loeb (1905, 1913), Harvey (1910, 1914), Elder (1912), and McClendon (1914), for the sea urchin, have expressed the view that the fertilization membrane forms after fertilization. Garrey (1919) is inclined to the belief that a membrane does not pre-exist on the unfertilized egg of *Asterias* and he has concluded that the formation of the fertilization membrane in the egg of *Arbacia* is *de novo*.

The differences in the phenomena involved in the development of the fertilization membrane among the various kinds of eggs have not been adequately realized. In many of the discussions on the subject, the attempt to generalize from one kind of egg has been made. From the contradictory conclusions it is obvious that the membrane on the sea urchin egg, if it pre-exists at all, is an exceedingly delicate structure. In many other kinds of eggs a definite, tough membrane pre-exists beyond doubt. Following activation this membrane is separated from the surface of the egg and develops into the fertilization membrane. This has been shown for *Chætopterus* (Whitaker and Morgan, 1930) and *Patiria* (Whitaker, 1931). The egg of *Urechis caupo* will be shown to be in the same category in the text to follow. The debatable cases are in the minority and include especially various species of echinids. It is shown in this work that the manner in which the fertilization membrane is developed differs radically for various kinds of eggs and that in every case the development of the fertilization membrane is actually a transformation of the vitelline membrane.

---

[1] This work was done during the tenure of a scholarship from Howard University, made available by a grant from the General Education Board.

MATERIAL AND METHODS

The experiments were done during the spring and summer of 1933 and the winter and spring of 1934 at Hopkins Marine Station, Pacific Grove, California.[2] Gametes were taken from four forms; *Dendraster excentricus, Strongylocentrotus purpuratus, Patiria* (*Asterina*) *miniata,* and *Urechis caupo.* These species were in abundance in the vicinity of the Station. Specimens were collected when needed. Sand dollars were obtained by dredging in Monterey Bay and sea urchins and starfish were taken at low tide from pools where the water was fairly deep. The large echiuroid *Urechis caupo,* described by Fisher and MacGinitie (1928, *a* and *b*), is an inhabitant of the mud flats of estuaries along the California coast. It lives in U-shaped burrows at a depth of about three feet and may be dug only when the flats are exposed. The worms live indefinitely when kept in aquaria with running sea water.

Ripe gametes of the two species of echinids were pipetted after the removal of a portion of the test. Only shed eggs of the starfish gave good results. Animals brought into the laboratory were spread on wet sea weed and they were induced to spawn in about an hour. The extruded eggs and sperm were pipetted off. Gametes of *Urechis* were drawn from the gonopores with small bore pipettes. All eggs were freed of bits of tissue, visceral fluid, and mucus and placed in finger bowls containing sea water. Dry sperm were placed in Syracuse watch glasses and sperm suspensions were made when needed by the addition of a drop of fresh dry sperm to 5 cc. of sea water. Glassware and instruments were sterilized before use and every precaution was taken to prevent the premature activation of eggs.

---

[2] The writer wishes to express his gratitude to Dr. D. M. Whitaker, Department of Zoölogy, Stanford University, who directed the work. He is also indebted to Dr. W. K. Fisher and the staff at the Hopkins Marine Station for their many courtesies.

Microscopic examination of eggs was made with a $1/12$ inch objective (Leitz) and a 20 × compensating ocular. For the cutting experiments, quartz needles were drawn to microscopic dimensions and mounted in a Taylor (1925) micromanipulator. Eggs were held in a hanging drop. For other microscopic manipulation of eggs micropipettes were made by drawing out lengths of quartz tubing so as to form capillaries at one end whose bores were nearly equal to half the diameter of the egg to be examined. A length of soft rubber tubing was attached to the large end of the pipette, the pipette rigidly clamped in position and supported on a ring stand, and so placed that the capillary end rested on a glass slide and in the field of the microscope. The free end of the rubber tubing was held in the mouth of the operator and by the exertion of a gentle suction eggs were handled and observed at the same time. In experiments where it was desirable to distribute cytoplasmic materials, an International Electric Centrifuge, Size No. 1, Type SB was used.

The solubility of the membranes on unfertilized and fertilized eggs was tested by exposure to a molar concentration of urea. Eggs were dropped in the urea solution and left for varying periods of time, then removed and observed under the high power of the microscope.

The Experiments

There is some doubt as to whether the peripheral layer of the unfertilized egg constitutes a definitely formed membrane in every case. The evidence which follows shows that for the four species which have been studied there is a superficial layer which is demonstrable by certain experimental methods. This layer will be shown to vary from a more or less rigid membrane to a soft, somewhat gelatinous material. Another question of importance is whether the membrane which develops after fertilization is identical with a preformed structure on the unfertilized egg. Observations were made on unfertilized and fertilized eggs in order to determine if possible the nature of the peripheral layer of the unfertilized egg on the one hand, and the origin and nature of the fertilization membrane on the other. Eggs were studied by microscopic examination, by the mouth manipulated micropipette, by the microdissection method, and by treatment with a molar concentration of urea. The experiments and results are presented.

Observations on the Development of the Fertilization Membrane

The normal manner in which the fertilization membrane arises in fertilized eggs of the sea urchin and the starfish varies so sharply that an untrained observer would note the difference. The following observations have been recorded for the purpose of emphasizing the difference found among eggs of the four forms studied.

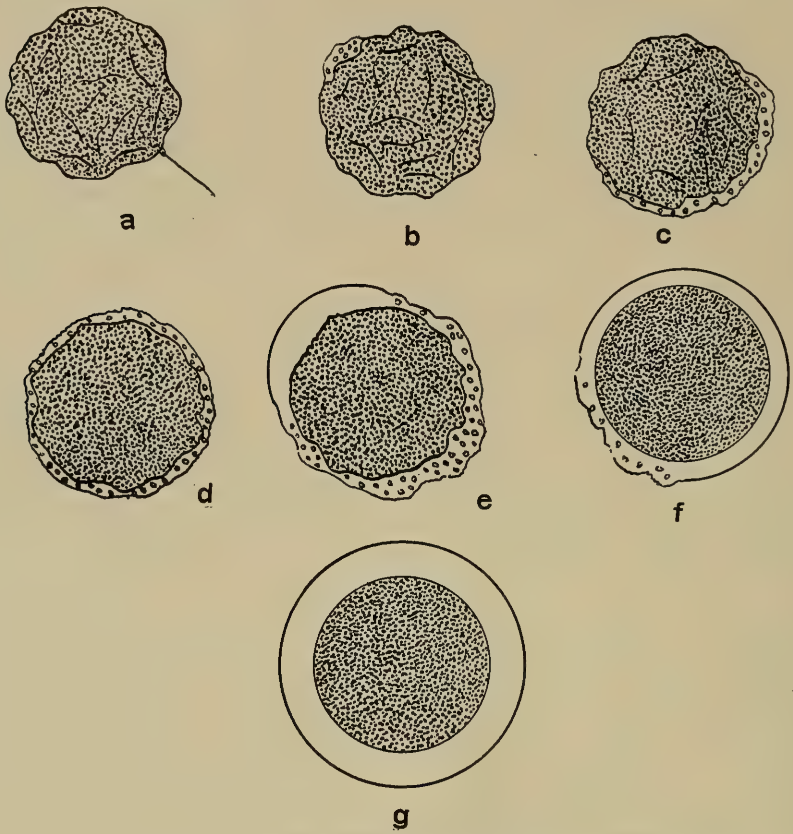


Fig. 1. Stages in the development of the fertilization membrane of the egg of *Dendraster* (egg jelly not shown). *a,* the sperm in contact with the egg about 5 seconds after insemination; *b,* 40 to 65 seconds after insemination the development of the fertilization membrane begins; *c,* spreading of the blister-forming process about 43 to 69 seconds after insemination; *d,* appearance of the egg 45 to 70 seconds after insemination. Note the tiny droplets as the perivitelline space forms, *b, c,* and *d.* Stage *e,* 75 to 100 seconds after insemination showing the initial thickening at the outer limit of hyaline formed when the blisters coalesced; *f,* progressive character of development of the thickened fertilization membrane. In *e* and *f* the tiny droplets disappear from the perivitelline space as the thickening of the fertilization membrane occurs. *g,* showing the fully developed fertilization membrane on the egg two and a half to three minutes after insemination.

Eggs of Dendraster excentricus.—Only mature eggs are shed by the sand dollar. They are nearly spherical in shape and prior to fertilization the surface is wrinkled. Eggs average about 120 microns in diameter and they are enclosed by a layer of jelly 70 to 80 microns wide. This jelly is studded with reddish pigment granules and the egg closely resembles that of the Atlantic coast sand dollar, *Echinarachnius parma*. A number of observations were made on the development of the fertilization membrane. All experiments and observations were made at a temperature of 15° C. Upon insemination with a thin sperm suspension in about 40 to 65 seconds a tiny blister appeared at a point in the circumference of the egg. This blister was bounded at its outer surface by a delicate film. The blister formation rapidly spread in all directions around the egg and it seemed that a hyaline substance flowed from the egg cortex, within which were scattered numerous minute droplets. Just (1919) reported similar droplets in the perivitelline space of the fertilized egg of *Echinarachnius*. The hyaline substance completely surrounded the egg in about five seconds, and the initial stage in the development of the fertilization membrane had taken place. About 75 to 100 seconds after insemination the film-like outer boundary of the hyaline substance had become thickened and the droplets had disappeared. The thickening began in the region where the first blister was formed and progressed over the entire outer limit of the hyaline material, accompanied by the simultaneous disappearance of the tiny droplets from the perivitelline space. In three to five minutes after insemination the development of the fertilization membrane was completed and the membrane stood equidistant from the egg surface at all points. Stages in the development of the fertilization membrane are shown in Fig. 1.

Eggs of Strongylocentrotus purpuratus.—Eggs of the sea urchin are smaller than eggs of the sand dollar, measuring about 80 microns in diameter. They are surrounded by a layer of transparent jelly 30 to 40 microns wide. The eggs are practically spherical with a smooth surface. Upon insemination at 15° C., in 30 to 40 seconds the egg surface was roughened. The roughening began at the point of sperm contact and in two to five seconds had spread around the egg in all directions. Closely following this a tiny vesicle was formed at the point where the roughening began and in a second the vesicle formation had spread around the egg. The vesicles fused and the egg became enveloped in a layer of hyaline substance, about 45 to 65 seconds after insemination. This marked the first stage in the development of the fertilization membrane. The next stage began with the thickening at the outer limit of the hyaline substance. The thickening began where the first vesicle was formed and it progressed around the egg so that in two to three minutes after insemination a rigid fertilization membrane was developed. This membrane stood out from the egg equidistant at all points and at no stage in its development could minute droplets be found in the perivitelline space. Stages in the development of the fertilization membrane are shown in Fig. 2.

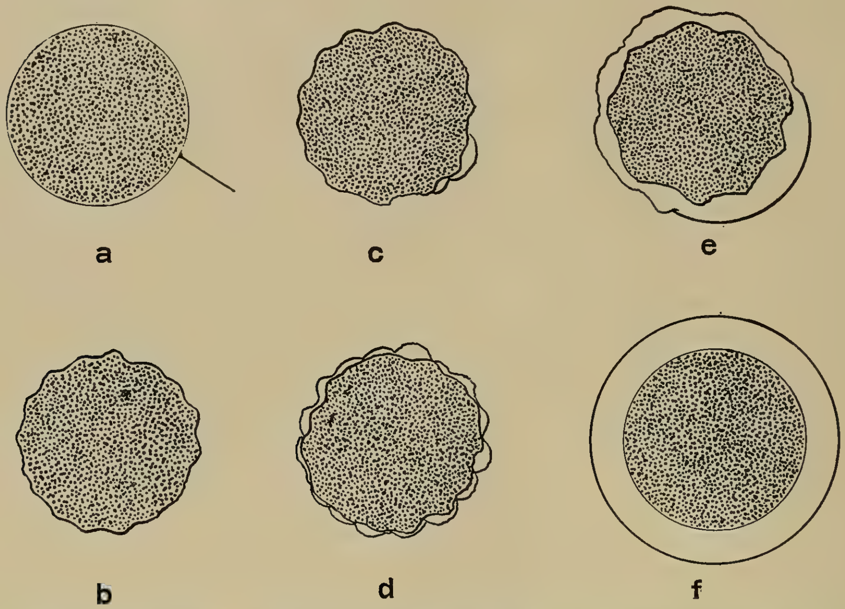


FIG. 2. Stages in the development of the fertilization membrane of the egg of *Strongylocentrotus purpuratus* (egg jelly omitted). *a,* sperm in contact with the egg about two seconds after insemination; *b,* roughening of the egg surface about 30 to 40 seconds after insemination; *c,* formation of a tiny vesicle to mark the initial stage in the development of the fertilization membrane, about 33 to 43 seconds after insemination; *d,* spread of the vesicle formation around the entire egg; *e,* beginning of the thickening of the outer portion of the hyaline material which composes the fused vesicles, about 37 to 47 seconds after insemination; *f,* the completely developed fertilization membrane about two to three minutes after insemination.

Eggs of Patiria miniata.—When eggs of this starfish are shed through the gonopores the germinal vesicles have just broken down. After about 10 or 15 minutes in sea water the first polar body forms. The mature unfertilized egg is larger (d. = 185 μ) than the sand dollar egg and it is enclosed by a definite, fairly tough membrane (Whitaker, *loc. cit.*). Eggs were inseminated at 15° C. and in 78 to 138 seconds the preformed membrane was separated from the underlying egg surface at one point. There was a flattening of the egg as the separation of the membrane began. The separation progressed slowly around the egg in all directions and in about three minutes the membrane was separated from the egg by a narrow perivitelline space which was gradually widened so that after about 10 minutes the fertilization membrane stood off from the egg at some distance. In many of the eggs which were studied it was found that the separating membrane remained in contact with the surface of the egg at one or more points for a considerable period after insemination. Hyman (1923) made similar observations on the lack of uniformity in the separation of the fertilization membrane in eggs of *Patiria.* Figure 3 shows stages in the separation and development of the fertilization membrane.

Eggs of Urechis caupo.—Eggs of this species are fertilized when

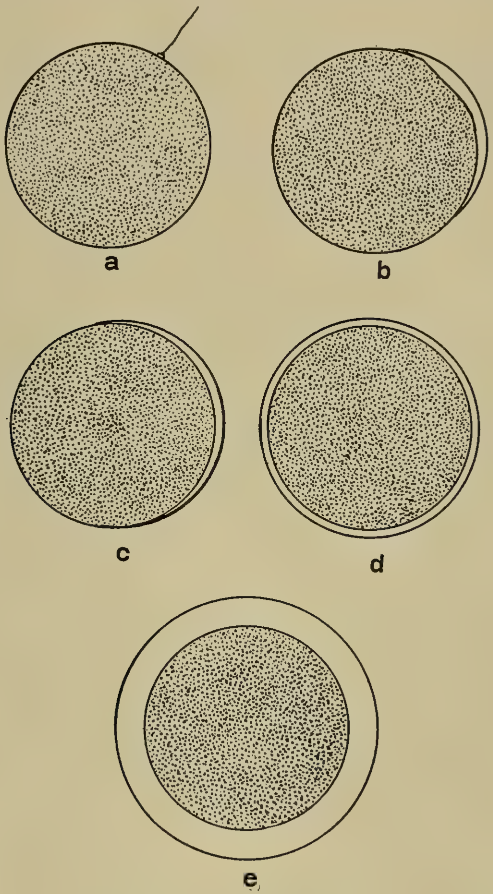


FIG. 3. Stages in the development of the fertilization membrane of the egg of *Patiria miniata* (egg jelly not shown). *a* shows the sperm in contact with the egg about 13 seconds after insemination; *b,* flattening of the egg surface and beginning of the separation of the vitelline membrane, 65 to 125 seconds after insemination; *c,* a later stage in the separation of the vitelline membrane; *d,* the membrane completely separated from the egg to become the fertilization membrane about 105 to 175 seconds after insemination; *e,* the fully developed fertilization membrane as it appears about 10 minutes after insemination.

they are in the germinal vesicle stage. The eggs are slightly smaller than eggs of the starfish, measuring about 130 microns in diameter. They are enclosed by a tough, pellicle-like membrane which is discernible by casual microscopic observation. Upon insemination at 15° C. the separation of the tough membrane began at the point of attachment of the sperm after about 8 to 15 minutes. The separation of the membrane was marked by a great wrinkling of the smooth preformed membrane. The separation of the membrane in large folds and wrinkles progressed around the whole egg. About five minutes after the separation of the membrane began, the irregular membrane was completely separated from the egg and in about a half hour after insemination this membrane was more regular and at a later period it was found to be smooth throughout and separated from the egg by a wide perivitelline space. Stages in the separation and the development of the fertilization membrane are shown in Fig. 4.

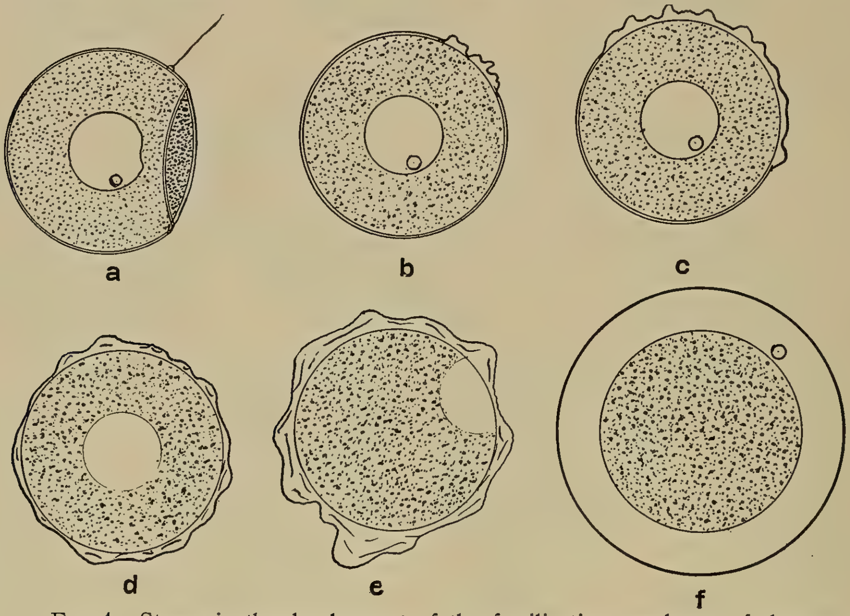


Fig. 4. Stages in the development of the fertilization membrane of the egg of *Urechis caupo*. *a* shows the sperm in contact with the egg about one and a half seconds after insemination; *b*, beginning of the separation of the vitelline membrane about eight and a half to fifteen minutes after insemination; *c*, the progressive character of the separation of the vitelline membrane; *d*, completion of the separation of the vitelline membrane to become the fertilization membrane, about twelve to twenty minutes after insemination; *e*, the appearance of the early fertilization membrane a few minutes later. In *b*, *c*, *d*, and *e*, note the wrinkled character of the separating vitelline membrane and of the early fertilization membrane. *f*, the fully developed fertilization membrane as it appears about thirty to sixty minutes after insemination.

Studies with the Micropipette

The microscopic examination of eggs with the best optical equipment offers little conclusive evidence of the nature of the surface layer of the unfertilized egg and of the fertilization membrane. For this reason certain experiments were devised for the examination of the superficial layer of the unfertilized egg and of the fertilization membrane during various stages in its development. A study by means of the micropipette is given below.

Eggs of Dendraster excentricus.—The surface of the unfertilized eggs was examined. The egg jelly was sucked off, its complete removal being indicated by the examination of the eggs in a solution of India ink. The removal of the jelly by this mechanical means did not interfere with the normal development of the fertilization membrane. When the mouth of the capillary was placed directly against the egg surface a portion of the egg was drawn into the capillary without producing a rupture of the delicate surface film. Such eggs were blown from the pipette and the part of the egg which had been drawn into the pipette appeared as an elongated stalk. They usually failed to cytolyze and when they were inseminated a fertilization membrane was developed which surrounded the entire egg and conformed to its irregular shape. This experiment showed that any preformed membrane on the unfertilized egg is capable of a great amount of extension, as has been shown by Just (1928) for uninseminated eggs of *Arbacia.*

The fertilization membrane was studied at various periods as it was developing. Eggs were caught at the mouth of the micropipette 40 to 60 seconds after insemination at which time the tiny blisters were forming. It was not possible to remove the hyaline substance composing the blisters from the egg. If the egg was sucked into the pipette so that only a small rounded mass of the clear substance was left at the mouth of the capillary and this mass examined with a microdissection needle, it was found to be soft and gelatinous. Even when the mass was held at the mouth of the pipette for a prolonged period its surface failed to thicken and harden so as to form a partial fertilization membrane. As a matter of fact, if such eggs were blown free of the capillary, the tough fertilization membrane failed to develop. Other eggs were examined 75 to 100 seconds after insemination when the thickening of the fertilization membrane had begun. When eggs were caught by the thickening portion of the developing membrane and this burst by suction, it was found to have become rigid and it failed to collapse. The ruptured edges of the membrane did not stick to the mouth of the pipette. If, however, the softer portion of the membrane was caught at the mouth of the capillary, it was ruptured and slowly collapsed. Its edges usually stuck to the pipette despite the fact that observations of this type were made on eggs from which the jelly had been removed. Other eggs were sucked from the developing fertilization membrane and the slowly collapsing membrane was left behind. The softer part of this membrane could be made to disintegrate by disturbance with a needle and only the more rigid portion could then be found. However, when eggs were examined three minutes or later after insemination, when the development of the fertilization membrane was complete, the membrane was no longer elastic and soft but it had become rigid and tough. If the egg were sucked out the membrane which was left behind failed to collapse.

From these observations it seems that there are three pronounced stages in the development of the fertilization membrane, namely, the cytolysis or sloughing off of a hyaline substance from the surface of the egg shortly after insemination; the progressive thickening at the outer limit of this substance at a slightly later period; and the development of a rigid, tough membrane some minutes after insemination.

Eggs of Strongylocentrotus purpuratus.—The surface of the unfertilized egg is bounded by a film. Attempts were made to suck the egg from this surface film as it appears that this film is the pre-existing membrane which has been described so often. This, however, was not possible. Eggs caught at the mouth of the micropipette were drawn into the capillary but no membrane was left behind. Other eggs were caused to cytolyze but no indication of a membrane could be found. Next, studies on the fertilization membrane were made. Eggs were examined about 40 seconds after insemination when the tiny vesicles were formed but they could not be sucked from the developing membrane. If the endoplasm was drawn into the pipette so that a mass of hyaline substance was left at the mouth of the capillary and this mass examined with a needle, it was found to be somewhat gelatinous. The outer surface of this mass failed to thicken and toughen and become transformed into a partial fertilization membrane. Other eggs were examined at the time when the thickening of the fertilization membrane began. This part of the membrane did not collapse when it was ruptured and its torn edges failed to stick to the pipette. When the soft part of the membrane was ruptured by the capillary, it partly collapsed and its edges stuck to the pipette. Eggs could be drawn from the developing fertilization membrane as in the case of the sand dollar egg. Membranes found on eggs three minutes after insemination or later were rigid and tough and they did not collapse when the egg was sucked from them.

Stages in the development of the fertilization membrane of the sea urchin egg comparable to the stages described for the egg of the sand dollar are indicated in the experiments.

Eggs of Patiria miniata.—There is no doubt that a tough membrane pre-exists on the unfertilized egg of the starfish. When unfertilized eggs were caught at the mouth of the capillary and sucked from the membranes and these latter examined with the needle, they were found to be very extensible. The membranes could be stretched considerably when they were caught between two needles. The empty membrane collapsed slightly when it was left in sea water. The fertilization membrane of the starfish egg was found to be less extensible than the membrane of the unfertilized egg. When the egg was sucked from it the fertilization membrane failed to collapse. There is no doubt that the membrane separated from the egg some minutes after fertilization was much more rigid and tough than the membrane which was found on the unfertilized egg.

Eggs of Urechis caupo.—The existence of a tough membrane on the unfertilized egg has been described above. Unfertilized eggs were caught at the mouth of the micropipette and the endoplasm sucked from the membrane, which remained slightly collapsed. Eggs were treated in the same manner at the time of the separation of the membrane and partly collapsed membranes remained. When eggs which had been fertilized for about a half hour were sucked from the developing fertilization membranes it was found that the rigid membranes failed to collapse.

The experiments with the micropipette revealed that the completely developed fertilization membrane differed in consistency from the vitelline membrane and from the fertilization membrane in its early stages of development.

Microdissection of Normal and Centrifuged Eggs

The vitelline membrane of the egg may be studied by the microdissection method. This method is also adaptable for a study of the fertilization membrane at different stages in its development. Experiments and the results of such studies are given.

Eggs of Dendraster excentricus.—Chambers (1921) reported the removal of a membrane from the surface of the unfertilized egg of the sand dollar, *Echinarachnius,* by tearing the membrane with a needle and slipping the egg out. The experiment was done with eggs which had stood for some time in sea water. I have attempted to repeat the experiment with the freshly shed egg of *Dendraster,* but without success. Eggs were held in a hanging drop of sea water and the tip of a very fine quartz needle was inserted beneath the peripheral layer. This layer was ripped by a stroke of the needle but in no case could the egg be slipped from a membrane. The ripping of the egg surface was either followed by the disintegration of the egg or by the formation of endoplasmic buds. Next the tip of the needle was passed just under the surface layer in such a way that the needle lay tangentially to the egg. By pulling the needle out from the egg, a hyaline substance was stretched and pulled outward with it. If the needle were withdrawn at this time, the hyaline material receded to the egg surface and some of it adhered to the tip of the needle. However, when the needle was pulled away from the egg to a considerable distance, the hyaline substance was drawn into a strand which snapped as the needle was pulled farther away. Some of the surface material receded to the egg and formed a nodular projection, while that which adhered to the tip of the needle rounded up and formed

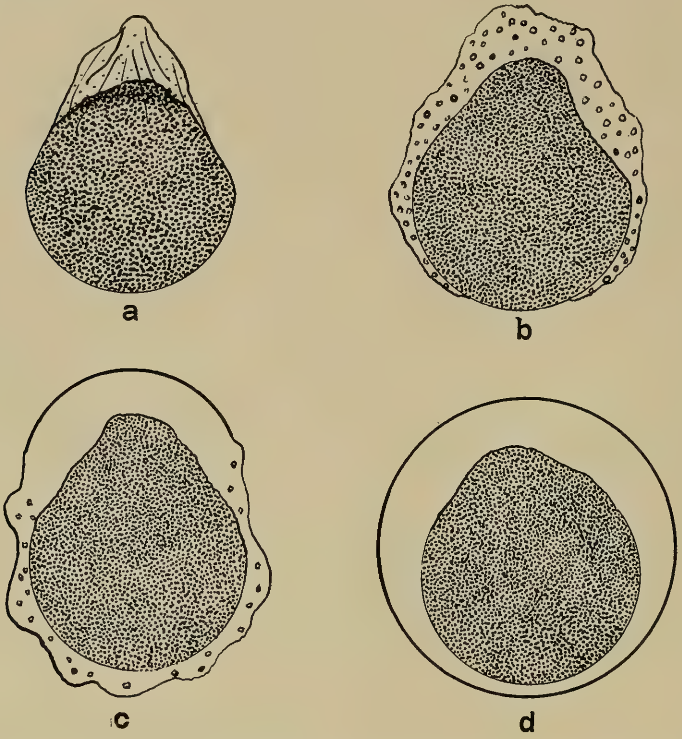


FIG. 5. Fertilization of centrifuged eggs of *Dendraster* (eggs centrifuged five minutes at 3,000 r.p.m.). *a,* appearance of the unfertilized egg immediately after centrifugation. *b,* initial stage in the development of the fertilization membrane in the egg about 40 seconds after insemination; *c,* thickening of outer portion of hyaline material about 20 seconds later than stage *b; d,* the completely developed fertilization membrane of the centrifuged egg, about three minutes after insemination.

a small globule. Complete fertilization membranes were developed when such eggs were fertilized.

The hyaline substance composed the peripheral layer of the unfertilized egg, and it was gelatinous and capable of great extension. It was not clearly differentiated from the underlying cytoplasm and its outer surface was bounded by a delicate film. It is possible that this film is identical with the membrane which many observers have described on the unfertilized egg. I believe that the entire superficial layer of hyaline material composes the vitelline membrane of the sand dollar egg. Only the outer boundary of this layer may be seen by ordinary visual means.

The development of the fertilization membrane is a transformation of the surface layer of hyaline substance from a soft, gelatinous state to the tough, rigid membrane. To what extent the normal development of the fertilization membrane could be modified by the centrifugation of the unfertilized egg seemed a question of interest. Eggs were centrifuged at various rates, the radius of centrifugation being 16.5 cm. When the unfertilized egg was centrifuged five minutes at 3,000 r.p.m., a cap of hyaline material was separated from the granular cytoplasm. Eggs were fertilized immediately after they had been centrifuged and a complete fertilization membrane was developed within which the egg lay eccentrically. (See Fig. 5*a*.) If the centrifuged eggs were allowed to stand in sea water for a few minutes before they were fertilized, the shifted material was redistributed and no effect was observed on the development of the fertilization membrane. Stages in the development of the fertilization membrane of the centrifuged egg are shown in Fig. 5. The cap of hyaline material was amputated and the two fragments formed were fertilized. Cuts were made in planes *AB* and *CD* as shown in Fig. 6*a*. All dissections were made immediately after eggs had been centrifuged. When the dissection was in plane *AB* one of the fragments was the egg proper and contained a large amount of granular cytoplasm, while the other fragment contained the hyaline material and a small amount of the endoplasm. The development of a fertilization membrane on the larger fragment was restricted to the cut surface while a complete membrane was developed by the smaller fragment. (See Fig. 6, *b* and *c*.) When the cut was in plane *CD* one of the fragments contained all granular cytoplasm and some of the hyaline substance and the other was composed of the hyaline material alone. Each was inseminated and a complete fertilization membrane was developed on the larger fragment while the smaller fragment was not affected by the sperm. See Fig. 6, *d* and *e*.

Other eggs were centrifuged for 14 minutes at 3,000 r.p.m. and the cap of hyaline material was greatly elongated and connected to the egg by a stalk of protoplasm, Fig. 7. Some of these eggs were fertilized immediately and the development of the fertilization membrane was restricted to the pole of the egg to which the hyaline substance was moved. The development of these eggs was followed through cleavage

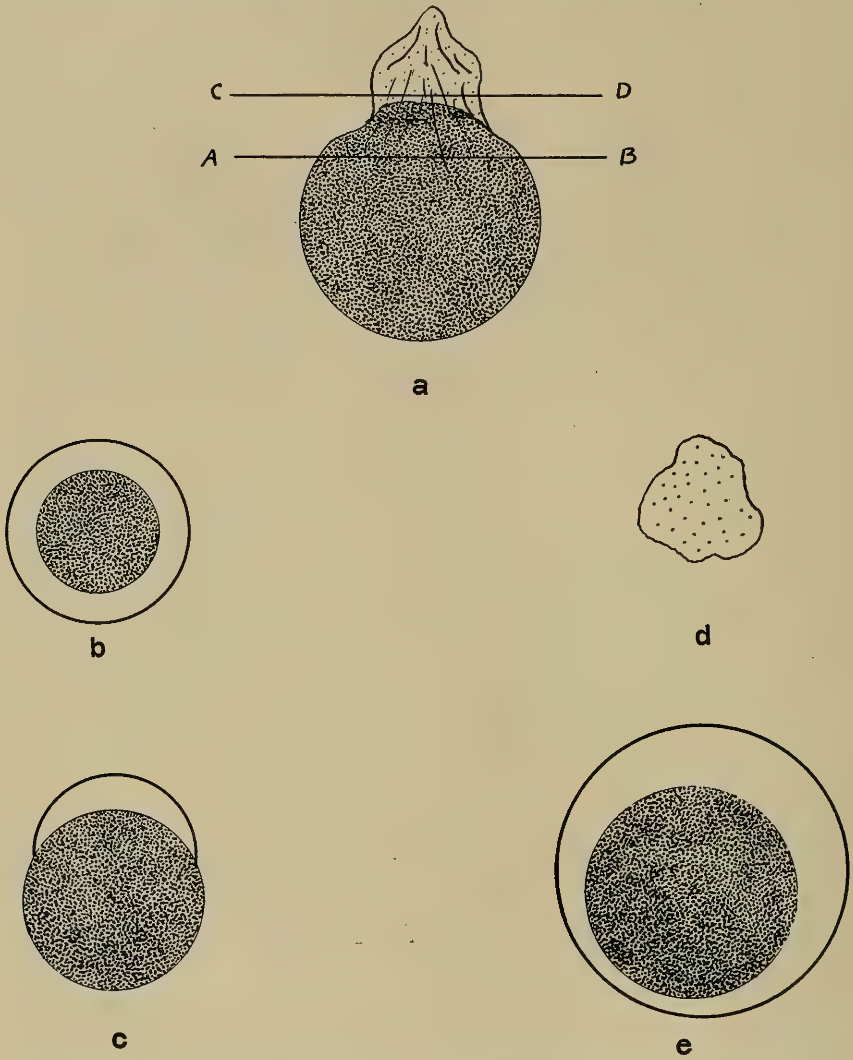


Fig. 6. Microdissection studies on centrifuged eggs of *Dendraster* (eggs centrifuged five minutes at 3,000 r.p.m.). *a,* planes of dissection in the centrifuged egg; *b* and *c,* development of the fertilization membrane on fragments formed when the egg is cut in plane *AB; d,* fragment containing hyaline material when the egg is cut in plane *CD,* and *e,* development of the fertilization membrane on a fragment when the egg is cut in plane *CD.*

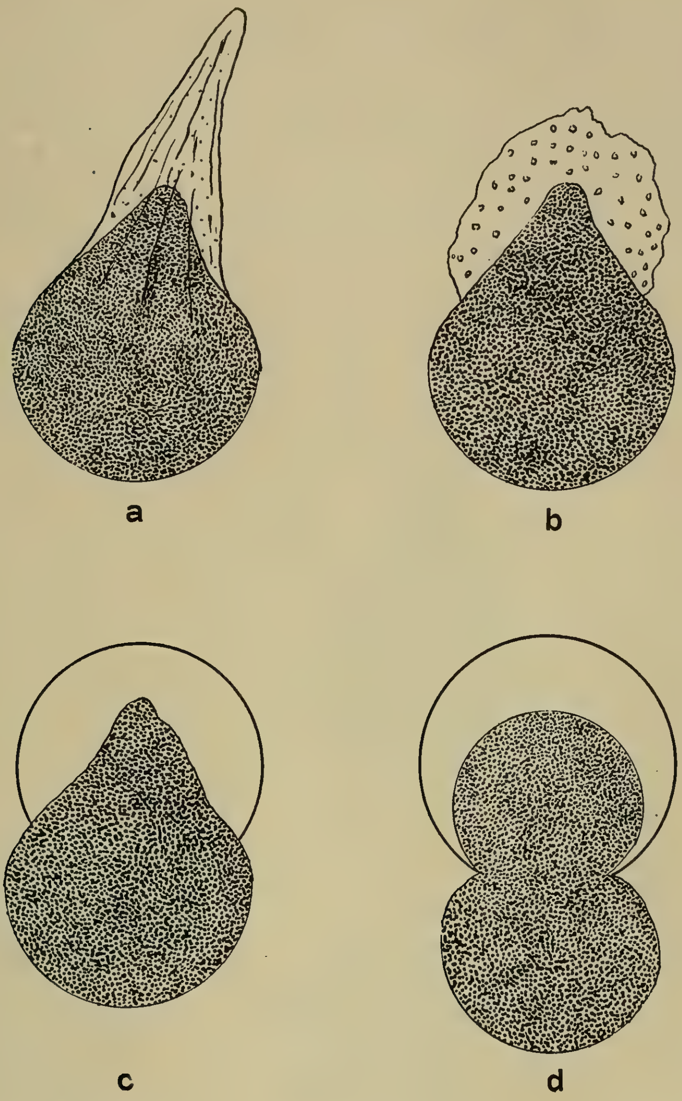


Fig. 7. Fertilization of centrifuged eggs of *Dendraster* (eggs centrifuged 14 minutes at 3,000 r.p.m.). *a,* appearance of the egg immediately after centrifugation; *b,* initial stage in the development of the fertilization membrane about 40 seconds after insemination; *c,* the completely developed fertilization membrane of about three minutes after insemination. *d,* first cleavage in the centrifuged egg, showing the fully developed fertilization membrane which lies around one of the blastomeres.

and it was found that one of the blastomeres lay within the fertilization membrane while the other was not enclosed by the membrane. Stages are shown in Fig. 7. The elongated cap of material was dissected from some eggs. The cut was made in plane *EF* (*see* Fig. 8*a*). One fragment contained a small amount of endoplasm and the hyaline material while the other consisted solely of granular cytoplasm. Each fragment was inseminated. The smaller of the two was unaffected by sperm and there was no development of the fertilization membrane about the larger fragment. The fragments are shown in Fig. 8.

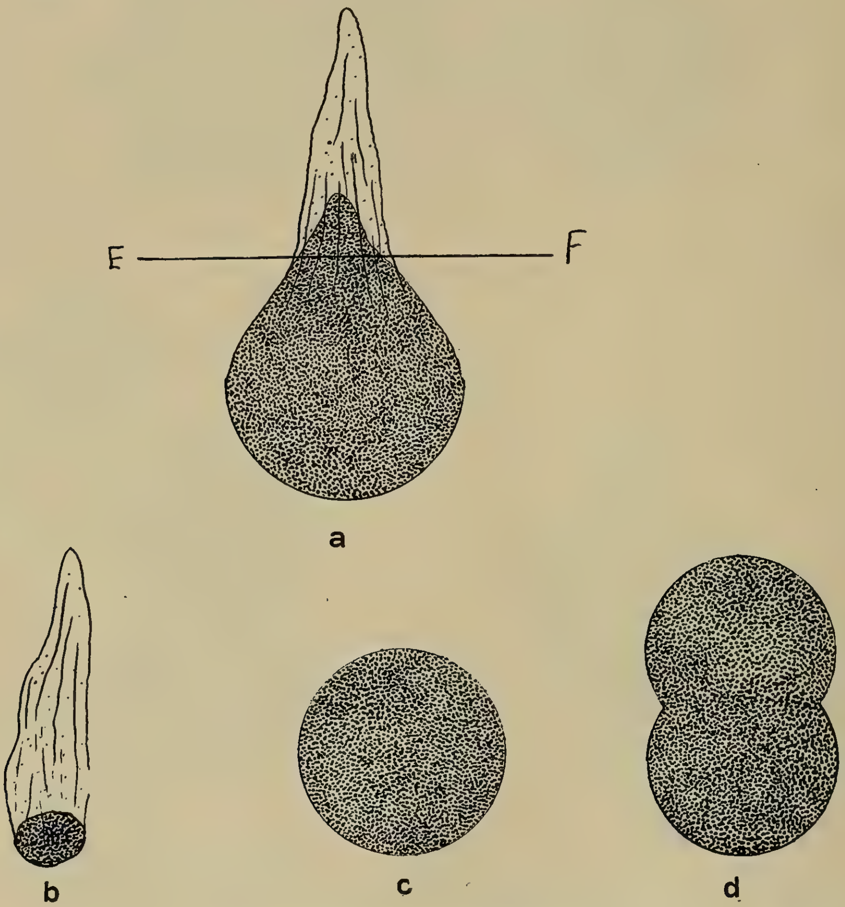


FIG. 8. Microdissection study on the centrifuged egg of *Dendraster* (egg centrifuged 14 minutes at 3,000 r.p.m.). *a,* the plane of dissection in the centrifuged egg; *b* and *c,* fragments formed when the egg is cut; each fragment was inseminated, the smaller being unaffected by the sperm and the larger failing to develop a fertilization membrane. *d,* cleavage of the larger fragment without the development of the fertilization membrane.

In the light of these experiments, it appears that a substance involved in the development of the fertilization membrane may be moved by centrifugalization. The only visible materials affected were the granular cytoplasm and the hyaline substance. It is significant that the fertilization membrane was found developing only about the portion where the hyaline substance was present. Examination of the substance with the microdissection needle revealed that it was similar to the gelatinous material which has been described as composing the peripheral layer of the unfertilized egg. There is no concrete evidence to support a presumption that these substances are identical, but, on the other hand, this fact is strongly suggested.

Eggs of Strongylocentrotus purpuratus.—The membrane on the unfertilized egg of the sea urchin, *Arbacia punctulata,* has been described by Kite (1912) and Chambers (1921). Chambers, repeating the observation of Kite says: " The needle is inserted as nearly as possible through the periphery of the egg and left there. Within a few seconds the protoplasm, lying immediately under the egg membrane and distal to the needle, flows away from the needle until the needle lies in a small protuberance which is formed by a very slightly lifted portion of the egg membrane." I repeated this observation and in my experiments the tip of a very fine quartz needle was inserted just beneath the surface of the egg. The granular cytoplasm receded gradually from the tip of the needle so that the point of insertion was marked by a hyaline zone bounded by a thin surface film. If the egg was held in position and the needle slowly pulled outward the gelatinous, hyaline material in which the needle tip lay imbedded was stretched. When the needle was pulled away far enough it was connected to the egg by a hyaline strand. As this strand was severed, part of it slowly drew back to the egg surface while that which was adherent to the tip of the needle formed a small clear droplet. I believe that the sea urchin egg is enclosed by a superficial layer of hyaline material and that this layer constitutes the vitelline membrane of the egg. The outer surface of this layer is a delicate film and the inner boundary is not differentiated from the underlying cytoplasm.

The membrane which develops after fertilization is at first soft and fragile but it becomes tough and rigid after a few minutes. These differences in the consistency of the fertilization membrane at various stages in its development have been mentioned above.

Attempts were made to separate the granular cytoplasm of the sea urchin egg from a hyaline substance as was done in the case of the egg of the sand dollar. Eggs were centrifuged at different rates, some of them as long as 30 minutes at 4,000 r.p.m., but when they were examined no such separation was found. These eggs were fertilized and apparently normal fertilization membranes were developed. High speed centrifugalization was tried. An air turbine ultra-centrifuge was used. Eggs were centrifuged for five minutes at approximate rates of 10,000 to 50,000 r.p.m. at a radius of 13.4 mm. Microscopic examination revealed that although the eggs were stratified, results similar to those observed with eggs of the sand dollar were not obtained. At higher rates of centrifugalization eggs were drawn out into bizarre dumb-bell shapes. Some of these were fertilized and, regardless of the peculiarity of the shape of the egg, a fertilization membrane was developed which completely surrounded the egg and conformed to its shape. It is possible that the hyaline material on the unfertilized egg is continuous with the underlying cytoplasm and thus could not be moved. At any rate it is capable of tremendous extension.

Eggs of Patiria miniata.—The membrane on the unfertilized egg of the starfish is a transparent, tough structure which remains intact though the enclosed egg may be divided into fragments as was done by Chambers (1921) for *Asterias* and Whitaker (1931) for *Patiria.* These experiments were repeated and confirmed in the present work. It was a simple matter to puncture the membrane of the unfertilized egg with the needle so that the endoplasm escaped and the partly collapsed membrane remained. When eggs were fertilized the probing of the developing fertilization membrane revealed that there was a progressive toughening of the membrane. If after some minutes the fertilization membrane was ruptured and the endoplasm permitted to flow out, the membrane which remained failed to collapse.

Eggs were centrifuged for a period as long as 30 minutes at 4,000 r.p.m. and when they were fertilized no effect on the development of the fertilization membrane was found. An interesting observation in this connection has been reported by Costello (1935), who found that when eggs of *Asterias* were centrifuged at certain periods during maturation there was a variation in the degree of the development of the fertilization membrane. He found that the separation of the fertilization membrane at the centripetal pole of the egg centrifuged 12 minutes at a force of approximately 6,000 times gravity was prevented. The formation of a thickened part of the fertilization membrane at the centrifugal pole of the egg and of a very thin part of the membrane at the centripetal pole was also mentioned. Costello concluded that eggs which were fertilized after a prolonged centrifuging failed to give a normal fertilization reaction with the separation of a normal fertilization membrane.

Eggs of Urechis caupo.—When eggs of this form were examined by the microdissection method it was found that the tough membrane was very resistant to cutting. The egg could be cut into fragments within its membrane without the parting of the membrane. When the membrane of the unfertilized egg was punctured with a needle and the endoplasm permitted to flow out, the partly collapsed membrane remained. If the membrane on the fertilized egg was punctured when the endoplasm escaped, the membrane which remained did not collapse. The early fertilization membrane was found to be softer and much more elastic than the fertilization membrane at a later stage. It was even softer than the membrane on the unfertilized egg. Within a half hour after the fertilization of the egg the separated membrane was transformed into a rigid, tough structure, as indicated by its resistance to the needle and its failure to collapse when the endoplasm flowed out.

Unfertilized eggs were centrifuged for periods as long as 30 minutes at 4,000 r.p.m. and then fertilized but no effect was observed on the development of the fertilization membrane. Taylor (1931) reported a greater thickness of the fertilization membrane at the centrifugal pole of *Urechis* eggs centrifuged 10 hours at a force of 4,800 times gravity.

The Effect of Urea on Egg Membranes before and after Fertilization

Certain physical changes in the nature of egg membranes have been described in other sections of this work. Tests were made of the solubility of egg membranes in an isosmotic solution of non-electrolyte. In the experiments a molar solution of urea was used. This concentration of urea is isosmotic with sea water and lacks the metallic ions which affect the properties of the egg membrane. A comparative study of the effect on egg membranes follows.

Eggs of Dendraster excentricus.—It was shown by Moore (1930) that fertilization membranes do not form on sand dollar eggs when the unfertilized eggs are bathed for a short time in an isosmotic solution of non-electrolyte and then fertilized. He found that urea in molar concentration dissolved off a delicate membrane from the unfertilized egg. Moore also found that the urea solution dissolved off the fertilization membrane of the sand dollar egg within the first five minutes after fertilization. He says, " If the eggs of the sand dollar within the first five minutes (if too long a time elapses the membrane becomes insoluble) after fertilization be put into a solution of non-electrolyte for 15 minutes the membrane will dissolve off the eggs." I have repeated and confirmed this observation on eggs of *Dendraster*. Unfertilized eggs were placed for two minutes in a finger bowl containing 50 cc. urea in molar concentration, removed, and placed in a second dish containing the same amount of the solution. After three minutes the eggs were removed from the urea, washed in sea water, and placed in dishes containing normal sea water. They were then inseminated but fertilization membranes failed to develop. Other eggs were fertilized, then placed in dishes containing 50 cc. of the urea solution at intervals of 30 seconds to 10 minutes after insemination and left in the solution for 15 minutes. It was found that the fertilization membrane dissolved off of all eggs placed in the urea up to two and a half minutes after insemination. In the other eggs the membrane had become insoluble. This experiment shows that the hyaline peripheral layer of the unfertilized egg (the fragile membrane described by Moore) are each affected in the same manner by the urea solution. The fertilization membrane of three minutes or later after insemination is radically different from any previous structure on the egg.

Eggs of Strongylocentrotus purpuratus.—Moore (1930) reported that the membrane of the unfertilized sea urchin egg could be dissolved off in a molar concentration of urea. The experiment was repeated and confirmed. Eggs were placed in 50 cc. of the solution and washed, after which they were transferred to a second dish containing the same amount of the solution. They were removed from this dish after two minutes, washed in sea water, then fertilized. The fertilization membrane failed to develop though the eggs cleaved later. When fertilized eggs were exposed to the same concentration of urea it was found that the fertilization membrane in its early stages of development was dissolved off. Membranes which had been developing for 30 seconds to two minutes were soluble in the urea solution after 10 to 15 minutes exposure. If, on the other hand, eggs were placed in the urea which had been fertilized for three minutes or more, the fertilization membrane was insoluble even after an exposure of 24 hours. The vitelline membrane of the egg and the fertilization membrane at an early stage in its development are affected in a similar manner by a molar concentration of urea, while the fertilization membrane at a later stage is insoluble in the urea and unlike any previous structure on the egg.

Eggs of Patiria miniata.—The membrane on the unfertilized egg of the starfish was insoluble in the urea solution even when given an overnight exposure. A half hour's exposure to urea is sufficient to cause the separation of the vitelline membrane from the egg. The fertilization membrane was also insoluble in the urea, showing a similarity in property to the vitelline membrane.

Eggs of Urechis caupo.—As was the case of the membranes of the starfish egg, both the vitelline membrane and the fertilization membrane in all stages of development were insoluble in a molar concentration of urea. It was found, however, that in the case of this egg all membranes could be dissolved off after an exposure of from 15 to 24 hours.

Discussion

It has been noted in the introduction that a widely debated question in the literature is whether the fertilization membrane forms by elevation and some modification of a membrane which already exists before fertilization as an investing structure in the egg cortex or whether it largely forms *de novo* as a product of the cortical reaction (e.g., by colloidal precipitation) and then elevates. In the debate upon this subject the diverse conclusions appear to apply almost exclusively to eggs of the sea urchin and the sand dollar. In most other forms the evidence, so far as it goes, indicates that a membrane pre-exists. It is generally agreed that the appearance of the fertilization membrane is in immediate sequence to the fertilization of the egg. The importance of the development of the fertilization membrane, in view of its relatively unimportant rôle in development, lies in its relation to and conspicuous part in the cortical reaction of the egg. Indeed, the development of the fertilization membrane in most cases is taken as criterion for the initiation of the development of the egg.

The phenomenon by which the fertilization membrane arises has been referred to in the literature as *membrane elevation, membrane formation,* or *membrane lifting;* which terms have been used interchangeably and without regard for the kind of egg considered. In my opinion it is preferable to speak of the *development* of the fertilization membrane. This is advisable when the following facts are considered: first, the fertilization membrane comes into existence only when eggs have been activated; second, in every case the fertilization membrane is developed as a result of the alteration of the superficial layer of the egg; third, the fully developed fertilization membrane is radically different from any previous structure on the egg and this difference is a progressive change which occurs during the time-lapse after the activation of the egg.

Let us consider the surface of the unfertilized egg. Beyond the vitellus is the more peripheral layer, which is called the vitelline membrane. This layer varies in consistency from a plastic, gelatinous substance in eggs of *Dendraster* and *Strongylocentrotus,* to a tough pellicle in eggs of *Patiria* and *Urechis.* Carter (1924), for *Sphærechinus granularis,* and Hobson (1932), for *Psammechinus miliaris,* have described the vitelline membrane as a hyaline substance whose outer boundary is a surface film and whose inner boundary is not differentiated clearly from the underlying cytoplasm. Chambers (1921), for the sea urchin and sand dollar, and Kite (1912), for the sea urchin, have demonstrated a tight fitting film-like membrane by the microdissection method. Thus it seems that the nature of the vitelline membrane is a matter of the interpretation of the observer. If the membrane is a delicate film which separates the egg from the jelly and sea water, the observations of Chambers and Kite must be accepted. If the membrane is a superficial layer of substance varying in depth and consistency, then it must be assumed that only the outer limit of the membrane hitherto has been demonstrated. The evidence presented strongly supports a concept that the vitelline membrane is a differentiated superficial layer of egg material which is not easily detected by ordinary visual means but whose existence may be shown in every case by the microdissection method.

The surface layer of the unfertilized egg (the vitelline membrane), while distinguishable from the underlying cytoplasm, is a part of the cortex of the egg. A large body of experimentation (Gray, 1922; Just, 1923, 1928a, 1928b; Lillie and Just, 1924, pp. 479 and 492–494), indicates that the egg cortex is the site of much of the physiological change which initiates the development of the egg. The fertilization membrane develops if activation is induced by artificial means instead of by the spermatozoön, and the extent of its development usually correlates with the effectiveness of the activation as revealed by the subsequent development of the egg. Loeb (1906, 1910, 1916) and Loeb and Wasteneys (1915) have presented much evidence supporting the view that essential changes in the cortex of the sea urchin egg which initiate development are cytolytic, resulting in increased oxidation and the formation of the fertilization membrane. The morphological changes (partial disintegration of the cortex and development of the fertilization membrane) closely parallel the physiological change in the egg. In the eggs which have been studied immediately after insemination certain changes take place in the egg cortex which are more marked in sand dollar and sea urchin eggs than in eggs of the starfish and *Urechis*. In the first two species the alteration of the egg surface is of a radical nature as indicated by the formation and coalescence of blisters as the surface lamella goes into dissolution. In eggs of the starfish and *Urechis* the portion of the cortex underlying the pre-existing membrane undergoes a partial disintegration and the surface layer (the egg membrane) becomes separated from the egg by a narrow space. The initiation of the development of the fertilization membrane is thus the result of the alteration of the superficial layer of cortical material. Whether the actual appearance of the membrane in its earliest stages is due to surface precipitation phenomena, as is believed by many writers, or to some other cause, cannot be said. In these experiments it is plainly indicated that the fertilization membrane originates from a pre-existing surface layer of the egg.

It is not possible to dissociate the fertilization membrane from its relation to the vitelline membrane. So intimate is this relation that the two membranes are considered by many workers to be identical. This assumption is not true. The vitelline membrane more correctly may be termed the *precursor* of the fertilization membrane. This fact is increasingly obvious in the light of observations on the development of the fertilization membrane. The results on the centrifuged eggs of the sand dollar represent an extreme example of the point in question. Among these the removal of a hyaline substance to a pole of the egg and the development of a fertilization membrane in this region of the egg, together with the failure of the eggs to develop membranes when the substance was amputated, produced strong evidence for the existence of a membrane precursor in the shifted material. Just (1923) has shown that the egg cortex is necessary for fertilization. The fact that the shifted material was cut away and eggs were fertilized with a resultant cleavage though the fertilization membrane failed to develop, revealed that the egg cortex was not damaged. Whether the hyaline material which was shifted by centrifugation was composed in large part of substance from the superficial layer of the egg cannot be said. One thing is certain, however, the presence of this substance is necessary for the development of the fertilization membrane. The localization of a membrane precursor by centrifugation in the other species of eggs was not demonstrated. Recently Costello (1935) has presented evidence on centrifuged eggs of *Asterias* showing that the development of the fertilization membrane usually was restricted to the centrifugal pole when eggs were centrifuged immediately after the breakdown of the germinal vesicle.

Physical and chemical changes in the properties of the fertilization membrane have been noted by many writers. Hyman (1923) and Chambers (1921) reported that the early fertilization membrane of echinoid eggs was considerably softer than the membrane at a later period. This has been found to be true of the membranes of the eggs which have been studied. The chemical changes which occur are discussed in the literature and they have been reviewed by Garrey (1919) and Hobson (1932). These changes may be correlated with definite stages in the development of the fertilization membrane. It is shown in this work that the vitelline membrane of the egg of the sand dollar and the egg of the sea urchin are soluble in a molar concentration of urea and likewise the fertilization membrane at certain stages in its development. Membranes on eggs of the starfish and the worm, *Urechis*, are insoluble in this solution.

A study of the origin and nature of the fertilization membrane reveals that eggs fall in at least two general categories. Each category is characterized by the nature of the vitelline membrane and the manner in which the fertilization membrane is developed. Eggs of *Dendraster* and *Strongylocentrotus* fall in one group. In these, as has been pointed out above, the fertilization membrane is developed from a flexible, somewhat gelatinous superficial layer of hyaline material which is easily demonstrated on the unfertilized egg. In the other group are found eggs of *Patiria* and *Urechis;* among these the peripheral layer of the unfertilized egg is a pellicle-like membrane which following fertilization is separated from the egg as the underlying surface undergoes dissolution. This separated membrane is developed into the fertilization membrane.

SUMMARY

The morphological changes in the development of the fertilization membrane have been described and figured in the text.

1. Examination of the vitellus of the unfertilized egg by means of the micropipette and by the microdissection method indicate that a surface layer (the vitelline membrane) is demonstrable on eggs of the forms studied. For *Dendraster* and *Strongylocentrotus* the superficial layer is a soft, flexible, gelatinous substance. In contrast to this, eggs of *Patiria* and *Urechis* are enclosed by a definite tough membrane.

2. In every case the fertilization membrane is developed from the superficial layer of the unfertilized egg. In the sand dollar and sea urchin this takes place by a radical transformation and alteration of the surface layer. For the starfish and *Urechis* the process is essentially a separation of the tough outer layer (the egg membrane) and its transformation into the fertilization membrane.

3. The superficial layer of the unfertilized egg properly may be considered a precursor to the fertilization membrane. This is splendidly shown in the microdissection and centrifuge studies on eggs of the sand dollar where the development of the fertilization membrane is greatly modified.

4. Changes in the consistency and properties of the fertilization membrane occur. These changes may be correlated with stages in the development of the membrane. They offer further substantial proof that the vitelline membrane and the fertilization membrane are not identical.

5. That the fertilization membrane is actually a structure which is developed as a consequence to the activation of the egg is shown by its difference from any pre-existing structure on the unfertilized egg.

6. From the evidence presented it is concluded that eggs fall into two general categories, namely, a group in which the fertilization membrane is developed in large part from a pliable superficial layer of substance; and a group in which the fertilization membrane is developed by the separation from the egg of a tough pre-existing membrane.

BIBLIOGRAPHY

CARTER, G. S., 1924. On the Early Development of the Echinoderm Egg. I. The visible changes following fertilisation and artificial parthenogenesis on the surface of the egg of Sphærechinus granularis. *Proc. Camb. Phil. Soc., Biol. Sc.,* **1**: 79.

CHAMBERS, ROBERT, 1921. Microdissection Studies. III. Some Problems in the maturation and fertilization of the echinoderm egg. *Biol. Bull.,* **41**: 318.

COSTELLO, D. P., 1935. Fertilization Membranes of Centrifuged Asterias Eggs. I. The effects of centrifuging before fertilization. *Physiol. Zool.,* **8**: 65.

ELDER, J. C., 1912. The Relation of the Zona Pellucida to the Formation of the Fertilization Membrane in the Egg of the Sea-Urchin (Strongylocentrotus purpuratus), *Arch. f. Entw.-mech.,* **35**: 145.

FISHER, W. K., AND G. E. MACGINITIE, 1928a. A New Echiuroid Worm from California. *Ann. and Mag. Nat. Hist.,* Ser. 10, **1**: 199.

FISHER, W. K., AND G. E. MACGINITIE, 1928b. The Natural History of an Echiuroid Worm. *Ann. and Mag. Nat. Hist.,* Ser. 10, **1**: 204.

FOL, H., 1877. Sur le commencement de l'hénogenie chez divers animaux. *Arch. de Zool. Expér. et Gén.,* **6**: 145.

FOL, H., 1879. Recherches sur la fécundation et le commencement de l'hénogènie chez divers animaux. *Mem. d. Soc. d. Phys. et d'Hist. Natur. de Genève,* **26**: 89.

GARREY, W. E., 1919. The Nature of the Fertilization Membrane of Asterias and Arbacia Eggs. *Biol. Bull.,* **37**: 287.

GLASSER, O. C., 1914. On Auto-parthenogenesis in Arbacia and Asterias. *Biol. Bull.,* **26**: 387.

GRAY, J., 1922. A Critical Study of the Facts of Artificial Fertilization and Normal Fertilization. *Quart. Jour. Micr. Sci.,* **66**: 419.

HARVEY, E. N., 1910. The Mechanism of Membrane Formation and other Early Changes in Developing Sea Urchins' Eggs as Bearing on the Problem of Artificial Parthenogenesis. *Jour. Exper. Zool.,* **8**: 355.

HARVEY, E. N., 1914. Is the Fertilization Membrane of Arbacia Eggs a Precipitation Membrane? *Biol. Bull.,* **27**: 237.

HERBST, C., 1893. Ueber die künstliche Hervorrufung von Dottermembranen an unbefruchteten Seeigeleiern nebst einigen Bemerkungen über die Dotterhautbildung überhaupt. *Biol. Centralbl.,* **13**: 14.

HERTWIG, O. AND R., 1887. Über den Befruchtungs- und Teilungsvorgang des tierischen Eies unter dem Einfluss äusserer Agentien. *Jena. Zeitschr.,* **20**: 120.

HOBSON, A. D., 1927. A Study of the Fertilisation Membrane in the Echinoderms. *Proc. Roy. Soc. Edinb.,* **47**: 94.

HOBSON, A. D., 1932. On the Vitelline Membrane of the Egg of Psammechinus miliaris and of Teredo norwegica. *Jour. Exper. Biol.,* **9**: 93.

HYMAN, L. H., 1923. Some Notes on the Fertilization Reaction in Echinoderm Eggs. *Biol. Bull.,* **45**: 254.

JUST, E. E., 1919. The Fertilization Reaction in Echinarachnius parma. I. Cortical response of the egg to insemination. *Biol. Bull.,* **36**: 1.

JUST, E. E., 1923. The Fertilization Reaction in Echinarachnius parma. VI. The necessity of the egg-cortex for fertilization. *Biol. Bull.,* **44**: 1.

JUST, E. E., 1928a. Cortical Reactions and Attendant Physico-Chemical Changes in Ova Following Insemination. J. Alexander, New York, The Chemical Catalog Co., Inc., **2**: 567.

JUST, E. E., 1928b. Initiation of Development in Arbacia. IV. Some cortical reactions as criteria for optimum fertilization capacity and their significance for the physiology of development. *Protoplasma,* **5**: 97.

KITE, G. L., 1912. The Nature of the Fertilization Membrane of the Egg of the Sea Urchin (Arbacia punctulata). *Science,* N. S., **36**: 562.

LILLIE, F. R., 1919. Problems of Fertilization. The University of Chicago Press, Chicago, Illinois.

LILLIE, F. R., AND E. E. JUST, 1924. Fertilization, General Cytology. The University of Chicago Press, Chicago, Illinois.

LOEB, J., 1905. On Fertilization, Artificial Parthenogenesis, and Cytolysis of the Sea Urchin Egg. *Univ. Calif. Publ. in Physiol.,* **2**: 73.

LOEB, J., 1906. Versuche über den Chemischen Charakter des Befruchtungsvorgangs. *Biochem. Zeitsch.,* **1**: 183.

LOEB, J., 1910. How Can the Process Underlying Membrane Formation Cause the Development of the Egg? *Proc. Soc. Exper. Biol. and Med.,* **7**: 120.

LOEB, J., 1913. Artificial Parthenogenesis and Fertilization. The University of Chicago Press, Chicago, Illinois.

LOEB, J., 1916. The Organism as a Whole. G. P. Putnam's Sons, New York.

LOEB, J., AND H. WASTENEYS, 1915. Further Experiments on the Relative Effect of Weak and Strong Bases on the Rate of Oxidations in the Egg of the Sea Urchin. *Jour. Biol. Chem.,* **21**: 153.

McCLENDON, J. F., 1914. On the Nature and Formation of the Fertilization Membrane of the Echinoderm Egg. *Intern. Zeitschr. Phys. Chem.,* **1**: 163.

MOORE, A. R., 1930. Fertilization and Development without Membrane Formation in the Egg of the Sea Urchin, Strongylocentrotus purpuratus. *Protoplasma,* **9**: 9.

MOORE, A. R., 1930. Fertilization and Development without the Fertilization Membrane in the Egg of Dendraster excentricus. *Protoplasma,* **9**: 18.

TAYLOR, C. V., 1925. Improved Micromanipulation Apparatus. *Univ. Calif. Publ. Zool.,* **26**: 443.

TAYLOR, C. V., 1931. Polarity in Normal and Centrifuged Eggs of Urechis caupo Fisher and MacGinitie. *Physiol. Zool.,* **4**: 423.

WHITAKER, D. M., 1931. On the Conduction of Cortical Change at Fertilization in the Starfish Egg. *Biol. Bull.,* **60**: 23.

WHITAKER, D. M., and T. H. MORGAN, 1930. The cleavage of Polar and Antipolar Halves of the Egg of Chætopterus. *Biol. Bull.,* **58**: 145.

THE FERTILIZABLE PERIOD OF THE EGGS OF FUNDULUS HETEROCLITUS AND SOME ASSOCIATED PHENOMENA[1]

BENJAMIN M. KAGAN

(*From the Marine Biological Laboratory, Woods Hole, Mass.*)

The longest time that an egg in a specified medium remains responsive to insemination by yielding normal cleavages is spoken of as the fertilizable period of that egg. The structural changes and their relation to the fertilizable period of the egg of the brackish water minnow, *Fundulus heteroclitus,* are discussed.

The literature dealing with the embryology of *Fundulus* lacks uniformity in regard to the names for the different structures. The terminology used here is that which is common to most of those who have dealt with morphology. (Kellicott, General Embryology, 1913.) The membrane which is normally separated soon after fertilization is the vitelline membrane. Between the vitelline membrane and the cytoplasm is the perivitelline space. The vitelline membrane has also been called the fertilization membrane, a term which may be misleading since fertilization is not necessary for its separation. The jelly substance which surrounds the vitelline membrane is termed the chorion. The platelets are disc-formed structures lying in the outer plasma layer of the egg.

Comparatively little work has been reported upon the initiation of development in the eggs of *Fundulus heteroclitus.* Stockard (1907) mentions the fact that some unfertilized eggs develop blastodiscs. No one has reported that unfertilized eggs in sea water will develop a perivitelline space, that their platelets disappear and blastodiscs form.

The papers of Loeb give the impression that sperm will react with artificially activated eggs in an entirely normal manner. Subsequent investigation on the same and other species and the present work on *Fundulus* show that such is not the case. Once development has begun in the *Fundulus* egg, fertilization is impossible.

Newman (1905), who reported some of the earliest experimental work on *Fundulus,* says that the change in the character of the membrane must involve an alteration in surface tension that controls the entrance of the sperm. He showed that calcium salts are especially active in preventing fertilization and suggests that they function by precipitating the colloids of the "egg membrane." No microscopical observations seem to have been made beyond that necessary to deter-

---

[1] This work was begun on the Edwin Linton Scholarship of Washington and Jefferson College in the embryology class of Woods Hole during the summer of 1933, and continued during the summer of 1934.

mine the percentage of cleavage. It is now known that the vitelline membrane is permeable to the calcium ion and therefore one can not be certain whether the locus of the effect is in the vitelline membrane or in the cortex or cytoplasm of the egg.

Wilson Gee (1916) studied the effects of alcoholization on *Fundulus* eggs prior to insemination and clearly demonstrated that profound effects may be produced upon freshly laid eggs by means, which in later stages, would have but slight effect. This may be easily demonstrated by the use of common preservatives for these eggs.

Carl Moore (1916), using butyric acid as activator, showed that the fertilization capacity of *Arbacia* eggs falls off gradually as the percentage of membrane production increases. The results which have been found for *Fundulus* show a similar relation between the percentage of fertilization and the percentage of eggs in which the perivitelline space has formed by the action of sea water.

This investigation was conducted under the direction of Dr. H. B. Goodrich, to whom I am deeply indebted. I wish to acknowledge also the valuable assistance which Dr. George L. Streeter gave in the preparation of the paper.

Material and Methods

The material used in this investigation was restricted to the brackish water minnow, *Fundulus heteroclitus,* of the Woods Hole region. The great majority of experiments were begun on the day following seining, the fish being kept meanwhile in the laboratory live car or in the running sea water in the laboratory aquaria. The procedure was as follows: Male and female fish were separated and the female fish were placed in running fresh water for twenty minutes prior to stripping to insure non-fertilization of the eggs obtained.

As much as possible, experiments were performed upon eggs from a single female. It is not rare during June and July to obtain fish with as many as 800 mature eggs. About 100 eggs were stripped into each of a series of bowls. Each bowl had previously been filled with about 50 cc. of sea water. One lot was inseminated in just enough water to cover the eggs and five minutes later 50 cc. of sea water was again added to the lot. At intervals of five or more minutes after stripping, other lots were inseminated in like manner. One control lot was not inseminated. As much as possible, eggs were kept from sticking together by running teasing needles between them. The fact that a different male must be stripped of milt for each lot may introduce a variability factor. Furthermore, no precaution can be taken in this type of procedure to standardize efficiently the quantity of sperm used.

In experiments in which different females were used, the lots of eggs were obtained at 5-minute intervals. Thus, each lot had eggs from a different individual. At the proper time, milt was added to all lots at once. Attempts to standardize the quantity of sperm by adding to each lot equal quantities of a fresh milt suspension gave less uniform results and the percentage of fertilization even in the lots which were inseminated immediately was lower than in the former type of procedure.

Three to three and a half hours after insemination all but about 1 per cent of the eggs which were going to cleave had cleaved and their further development was arrested by the use of Stockard's solution. It was convenient to stop the cleavages at this time because the distinction between the blastodiscs and early cleavage stages was much clearer then than when the blastomeres became very small. When counting eggs, 25 to 50 were placed in a depression slide whose depth was very slightly less than the average diameter of the eggs. By moving a cover glass over the surface of the depression, it was possible to turn the eggs over so that observations of any part of the egg could be made.

Before each experiment was begun the pH (indicator methods), specific gravity, and temperature of the water to be used were recorded. The pH of sea water was constantly 8.4 and the specific gravity 1.020.[2] The effect of the slight temperature variations or seasonal variations, if any, were masked by the wide variation in the eggs themselves.

The Effect of Delayed Insemination upon the Percentage of Eggs Fertilized

The vexing problem of a low yield of fertilized eggs has at some time confronted every investigator working with *Fundulus*. Dry fertilization was recommended in order to remedy this situation (Stockard, 1906). At times it is more convenient and often better results are obtained if insemination is accomplished soon after stripping into just enough sea water to cover the eggs. Newman (1905) noticed that the eggs of *Fundulus heteroclitus* became incapable of fertilization if they were exposed to sea water for more than an hour. Gee (1916) remarked that there was a very much lowered percentage of fertilization in *Fundulus* eggs as a consequence of insemination which was delayed " some twenty to thirty minutes." No one, however, has reported just what is the exact condition.

The unfertilized egg of *Fundulus* has platelets and no perivitelline space. With fertilization, the space is formed, and the platelets disappear with the formation of a blastodisc which subsequently cleaves.

---

[2] The pH values given in this paper have not been corrected for the salt errors of the indicators or to a common basis of temperature.

Throughout this paper, the term *platelets* is used to refer to eggs which have platelets, no blastodiscs, and usually perivitelline spaces. *Nil* is a convenient term which is applied to an egg which has no platelets, no blastodiscs, is with or without a perivitelline space, and sometimes has no oil drops. Some of the eggs of this group which develop a perivitelline space and have oil drops may be regarded as being between the platelet and the blastodisc stages. Eggs have been observed to develop a blastodisc very soon after the disappearance of the platelets and it may be that these eggs have been observed in this intermediate stage. Others, mostly lacking oil drops, are considered defective eggs and are found in but few numbers. The *nil* eggs are not immature eggs because they are equal or occasionally larger in size and more transparent than mature eggs, whereas immature eggs are smaller than mature ones and are opaque. The term blastodiscs refers to those eggs in which platelets are absent; blastodiscs which do not cleave are formed and perivitelline spaces are present. Cleavage is characterized by a cleaved blastodisc, perivitelline space, and the absence of platelets. The protocol of a typical experiment follows:

Experiment 28, June 19, 1934. Sea water analysis pH 8.4 (cresol red). Specific gravity 1.020. Temperature 19.8° C.

10:05 A.M.	Eggs of one female distributed into seven bowls, each of which contains 50 cc. of sea water.
10:06	Water is poured off Lot 1, leaving the eggs barely covered with water, and they are inseminated with fresh sperm, mixed and separated from each other by teasing needles.
10:10	The same is done to Lot 2, and 50 cc. of water is added to Lot 1.
10:15	The same is done to Lot 3 and Lot 2 respectively.
10:20	The same is done to Lot 4 and Lot 3 respectively.
10:25	The same is done to Lot 5 and Lot 4 respectively.
10:30	The same is done to Lot 6 and Lot 5 respectively.
10:35	50 cc. of sea water is added to Lot 6. Lot 7 remains as uninseminated control.
1:00 P.M.	Stockard's solution is used to replace the water in all bowls.

Figure 1 represents the data taken from the experiment described above. There is a distinct drop in the percentage of fertilization (cleavages) obtained between the 10 and 15-minute treatments, which seems to indicate an important change occurring in most of the eggs at that time. The relation between the curves of blastodiscs and cleavages and reference to the uninseminated control show that a fertilizable egg will develop a blastodisc in sea water if it is not fertilized. The control shows also that while uninseminated eggs develop blastodiscs in sea water, they will not cleave and that those which do not produce a blastodisc either maintain their platelets or belong to the defective *nil* group described above.

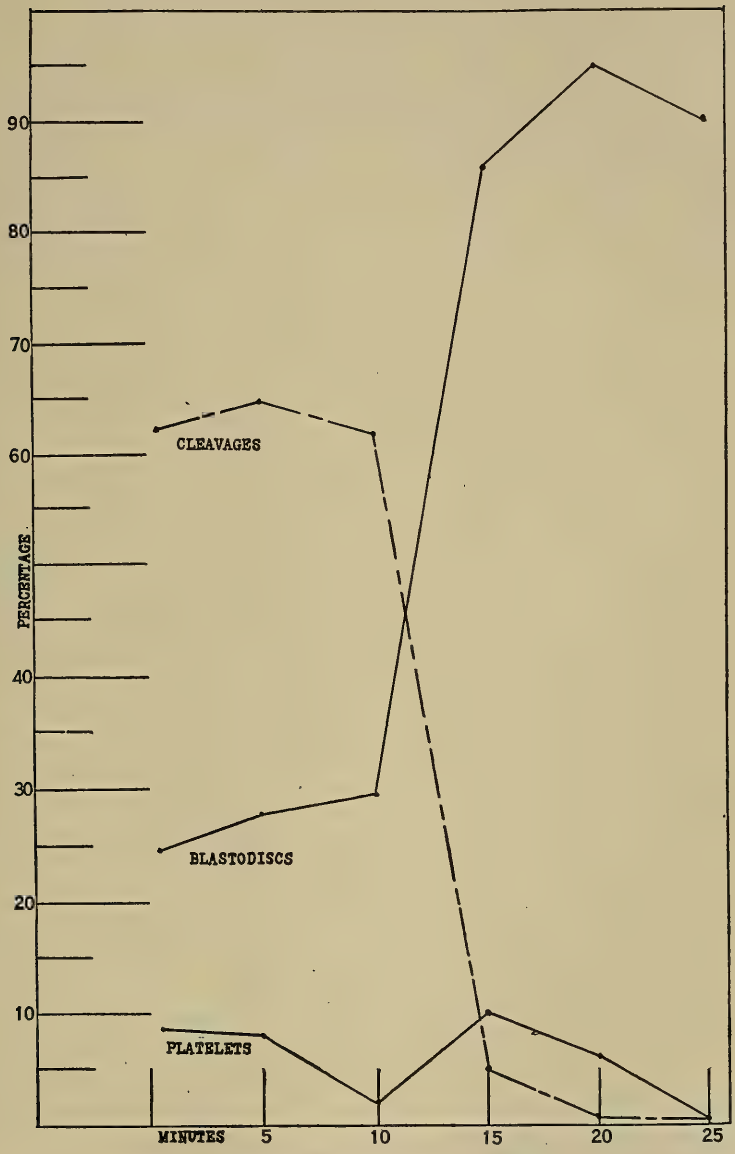


Fig. 1. Condition of eggs three hours after stripping. The time that the eggs were exposed to sea water prior to insemination is indicated by the horizontal distance; vertical distance represents the percentage of eggs that showed respectively the three named stages: platelet, blastodisc, and cleavage.

In Fig. 3, each curve represents the cleavage curve of a different experiment for which all the eggs used were taken from a single female. On each curve is marked the percentage of the total 56 experiments which that type of curve represents. That is, 5.82 per cent of the 56 curves were similar to curve I, 29.2 per cent of the total were similar to curve II, etc. The type of curve was determined by the shortest time at which less than 5 per cent of cleavages was obtained. Thus, a 25-minute curve indicates that eggs which are represented by that curve will give less than 5 per cent of cleaved eggs when insemination of the eggs in sea water is delayed for 25 minutes. Over 50 per cent of the curves are of the 15–20 minute variety, which have curves characterized by a drop which begins between 10 and 15 minutes. It has been concluded from the above data that the fertilizable life of the average *Fundulus* egg is from 15 to 20 minutes. In order, therefore, to obtain as high a percentage of fertilization as possible, it is recommended that *Fundulus* eggs be inseminated within fifteen minutes of stripping.

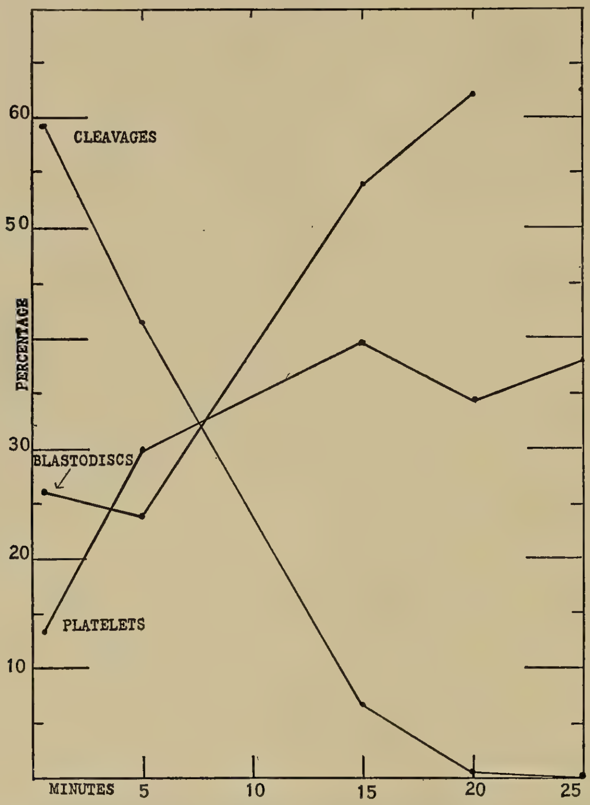


Fig. 2. Condition of eggs three hours after stripping. The time that the eggs were exposed to sea water prior to insemination is indicated by the horizontal distance; vertical distance represents the percentage of eggs that showed respectively the three named stages: platelet, blastodisc, and cleavage.

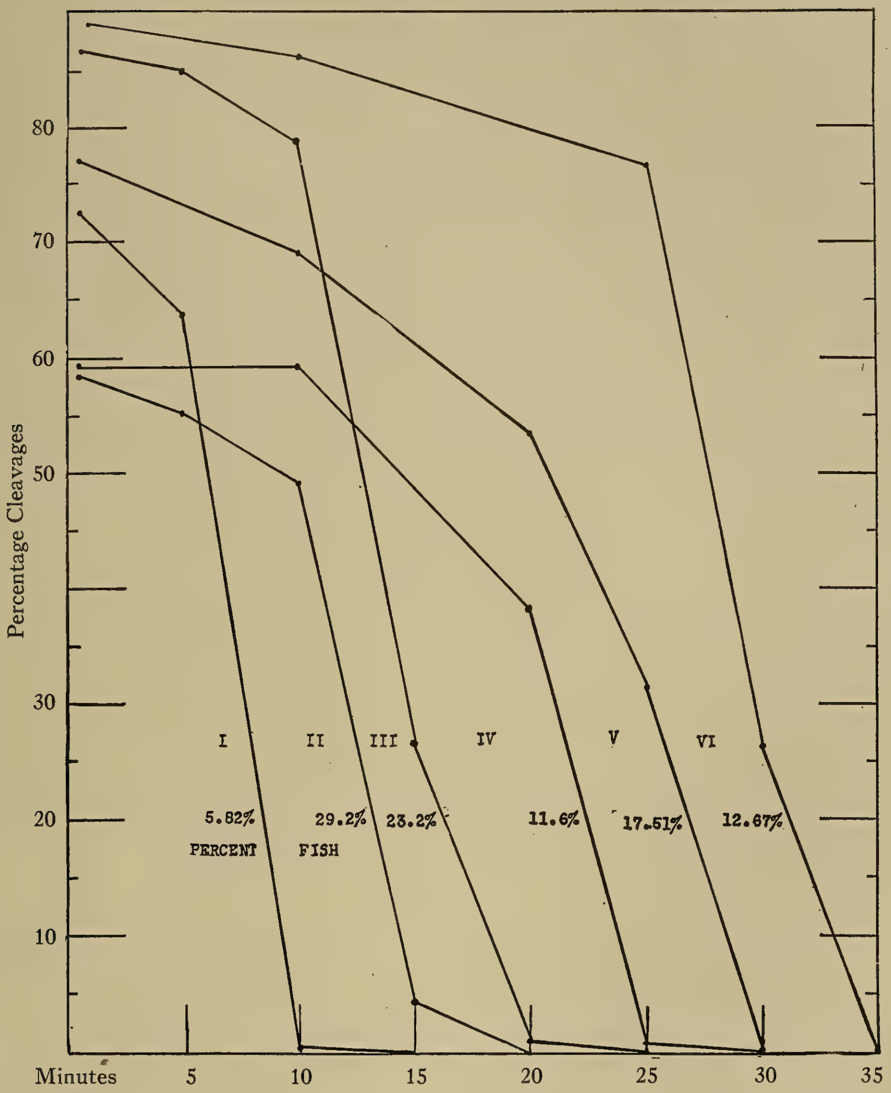


FIG. 3. Chart showing the effect of delayed insemination of eggs in sea water. The percentage of fertilized eggs varies according to the length of this delay. Of 56 fish studied, the eggs of each one were inseminated after different durations of exposure to sea water with changing rate of falling off in fertilization, thus producing the six types of curves illustrated. On each curve is labelled the percentage of the fish whose eggs gave results such as indicated by that curve. In about one-third of the fish (29.2 per cent), fertilization ceased at 20 minutes. However, a considerable proportion of the eggs obtained from 12.67 per cent of the fish were fertilized after 30 minutes exposure to sea water.

It is interesting that Gee (1916) showed the maximum life of *Fundulus* sperm to be 8 to 14 minutes. When *Fundulus* spawn, the milt is shot directly at the eggs as they are extruded. The eggs are said to have a strong chemotactic influence on the sperm and are sticky so that the sperm stick to them or are caught (Newman, 1907). Thus the eggs and sperm are influenced by the sea water for a minimum length of time before the eggs are fertilized.

Figure 3 shows to what extent the eggs of different individuals may differ. Figure 2 is presented for comparison with Fig. 1. Both represent experiments upon the eggs of a single individual which were performed in exactly the same manner. Note that the important difference appears to be in the percentage of platelets. The differences in the percentages of eggs stopping in the platelet stage characterize the entire series of graphs prepared in this way. A few experiments showed that if these eggs had been allowed to remain in sea water several hours longer, the platelet factor would be considerably but not completely reduced, and the percentage of blastodiscs proportionately increased. Since the absence of platelets in an egg is one of the first definite signs of activation by sea water, it is concluded that eggs vary considerably in the rate and susceptibility to activation by sea water. Eggs of different individuals vary so considerably that it was early found that experiments in which more than one female was used were valueless except that they convinced one of the variability factor. The wide variations reported by Wilson Gee (1916) are of a similar magnitude as those shown above. Referring to this situation in *Fundulus,* Newman (1908) says one " must learn to see the outline of fixity and regularity through the haze of a confusing diversity of conditions."

TABLE I

Results of Experiment 28 (described above)

Lot no.	Minutes in sea water prior to insemination	Number of eggs	Percentages of eggs showing			
			Platelets	Nil	Blastodiscs	Cleavages
1	1	102	8.5	4.7	24.5	62.3
2	5	115	7.9	0	27.6	64.5
3	10	106	1.6	7.5	29.5	61.4
4	15	119	9.9	0	85.8	4.3
5	20	123	5.6	0	94.4	0
6	25	114	0	10.5	89.5	0
7	(Control) not inseminated	124	6.5	4.1	89.4	0

In view of the variability of the eggs in sea water, many experiments such as reported above would be necessary to determine the extent of the effect of hypotonic sea water or similar solutions. Fresh and distilled water very definitely do not initiate development in as many eggs as does sea water. Most eggs in these solutions remain in the platelet stage, usually without developing a perivitelline space or a blastodisc. This shows that it is the sea water and not some egg substance such as fertilizin which activates the egg; or at least the egg substance plus sea water is much more efficient in activation than is the egg substance alone. This coincides with Just's (1930) criticism of the conclusions which Woodward (1918) drew from her work on *Arbacia* and *Asterias.* Since some eggs do develop perivitelline spaces in these solutions, one cannot conclude with the data at hand that this is merely an osmotic phenomenon. It is possible that further work with various salts and different concentrations of sea water may lead to additional significant data.

A Microscopical Study of Early Changes in the Egg System

Changes in the Unfertilized Eggs Coincident with the Loss of Fertilizability

In the investigation of this problem, it was necessary first to determine the time and character of the changes in the unfertilized egg in sea water. Eggs were stripped into a bowl of sea water by a procedure which was identical with that described above and another lot of eggs was inseminated properly as a control. Eggs for the two lots were always taken from one female because it was noted that while the eggs of different females did not differ greatly in the time required for the changes occurring after fertilization, they did differ somewhat in the time required for the changes which occurred through the action of sea water. Table II gives the results of a typical experiment of this series in which observations upon the eggs were made every five minutes up to two hours and at various intervals thereafter. The observations were made through the high power lens of a dissecting microscope and are therefore not of the utmost accuracy. It is noticed that the perivitelline space becomes pronounced in most unfertilized eggs between the 10 and 15-minute treatments. The following experiment was therefore planned to determine whether the development of this space is coincident with the drop in percentage fertilization which occurs at about this time:

A lot of about 50 eggs was allowed to remain in sea water for 15 minutes, whereupon the eggs were separated as quickly as possible into two lots. One lot consisted of eggs which at the time of observation appeared to have no perivitelline space; the other lot had a space. Both lots were then immediately inseminated. While the eggs were being separated into lots, some eggs which when observed had no space were probably developing a space. In spite of this, significant results were obtained. Cleavages developed in 64.8 per cent of those which had no space when observed. None of the eggs in the lot which had spaces ever cleaved. Lots of eggs which had been in sea water for 20, 25, and 30 minutes gave similar results. However, as the time of residence in sea water was prolonged, certain differences were noted which will be described later in this paper.

This experiment was also performed in another manner. The eggs of one female were stripped into two bowls containing sea water. Observations upon the eggs were made at 5-minute intervals. At 15 minutes, a few had developed the space, and one lot was immediately inseminated. Five minutes later, almost all the eggs in the other lot had developed perivitelline spaces and they were then inseminated. The first lot developed 69 per cent cleavages, the second lot but 16 per cent. It was concluded that those eggs which have developed a perivitelline space before insemination cannot be fertilized. Eggs have been observed which had not developed any space at the end of 30 minutes and were still incapable of fertilization. In these, the platelets usually remained and a blastodisc did not develop even after several hours in sea water.

TABLE II

Schedule of Events in Fertilized and Unfertilized Eggs in Sea Water at 20° C.

Minutes	Fertilized eggs	Unfertilized eggs
5	Platelets absent and complete perivitelline space present in almost all eggs.	
10		Small space present in a few eggs.
15		Perivitelline space is present in most eggs.
35		All eggs have developed spaces of varying size.
60		Cell platelets begin to disappear.
70	Blastodiscs present; all platelets have disappeared.	
90–130		Blastodiscs present; platelets present in some which have no blastodisc.
110	Two-cell stage.	
140	Four-cell stage.	
210	Sixteen-cell stage.	
260	Cleavage above count.	Very few eggs left with platelets.
19 hrs.	Germ ring.	Blastodiscs have disintegrated; globules separating out. Several eggs have retained platelets.

Observations on the Micropylar System during Fertilization and during Activation by Sea Water

The work on the capacity for fertilization after the initiation of development has been done mostly upon eggs which do not have micropyles. *Fundulus* appears to be no exception to the fact that teleost eggs generally do have micropyles. A funnel-like indentation in some freshly-laid eggs is particularly evident when it is in a semi-profile position. With the aid of an assistant, the following experiment was accomplished several times. A few eggs were stripped dry into the depression slides described in the section on material and methods. An egg was found as soon as possible which showed this indentation. A dilute sperm suspension which had been prepared meanwhile by the assistant was pipetted into the depression of the slide. The indentation of the egg surface was turned slightly away from under the cover-glass and then back again to the correct focus. The high dry lens of the microscope was then arranged to focus upon the center of this indentation. In this way, it was possible to see the actual passage of the spermatozoa into a few eggs. The sperm tail was not always in focus, but the head could easily be followed as it advanced to the center of the depression. Once it arrived there, a moment passed in which nothing seemed to happen. Even the sperm head did not seem to move. Then suddenly there was definite movement in the protoplasm just beneath the periphery of the egg. The beginning of the perivitelline space could be seen beneath the indentation and surrounding the center of it. As the cortex of the egg pulled away from the original egg surface, the sperm head seemed to be pulled through the center of the indentation. The sperm tail was lost among the threads of the chorionic jelly. The space spread quite rapidly around the surface of the egg by the further shrinkage of the yolk, and the indentation disappeared.

In unfertilized eggs a space also forms in the vicinity of the micropyle but the indentation persists for some time and finally seems to become deeper and narrower until the space has slowly spread around the rest of the surface of the egg, when the indentation begins to lose its depth and disappears. In a semi-profile view of this region of a freshly stripped egg, the author has observed a translucent threadlike strand stretching across the space between the yolk surface and the vitelline membrane after the perivitelline space had formed. As the space widened, the strand broke about one-third of the distance from the yolk surface and the remaining pieces were gathered up into the surfaces to which they had remained attached.

The time that these reactions take was not recorded, but it seemed that the reaction in the fertilized eggs was a matter of seconds and the reaction in the unfertilized eggs took a number of minutes. Once the vitelline membrane had definitely separated and a space which was well-defined under low power was visible, it was not found possible to demonstrate the penetration of sperm anywhere on its surface.

From these observations it is concluded that the formation of the perivitelline space involves the destruction of the micropyle system, and thereby prevents fertilization. It is quite evident that such a mechanism might be very efficient in preventing polyspermy.

Attempts were thereupon made to determine whether or not the sperm might initiate development if the block due to the vitelline membrane was removed, or if penetration by way of the micropyle is essential. By the use of a heavy microscope stand in the horizontal position with a mechanical stage to which a glass knife or needle was attached, it was possible to tear the vitelline membrane after its presence became noticeable under the high power of a dissecting microscope. About 35 minutes after stripping into sea water, the tear in the membrane was made. Fresh milt was immediately added while the tear in the membrane was kept patent by the needle. No cleavage was ever obtained in such eggs. An attempt was also made to inject sperm into the perivitelline space before it had attained full size but the results were always negative. However, it was found impossible to fertilize freshly stripped eggs with milt which was forced through such a fine glass injection needle as was being used. Professor Chambers has also observed the fact that spermatozoa, particularly of echinoderms, lose their power to fertilize when they have been forced through such a needle. He has offered as explanation (personal communication) that the effect is probably due to the loss of motility of the spermatozoa by the agglutination of their tails while they are crowded together in a narrow channel. It is hoped that the technique for removing the membrane entirely, which was developed by Nicholas (1927), may aid in future work in this direction. To determine whether or not it is essential for the sperm to approach the egg by the micropyle even after the removal of the vitelline membrane, it will be necessary to remove it immediately after its formation and before the egg itself has changed very much. It is difficult to see how even this technique will accomplish the task without injuring the egg cortex.

The formation of the perivitelline space in *Fundulus* seems to be essentially a cortical change. The vitelline membrane does not rise off the yolk as it does in some forms but rather the yolk shrinks from the membrane, an event which necessarily involves a change in the permeability of the cortex. This is a phenomenon which is easily observed, since the diameter of the whole egg remains rather constant while the vitellus proper becomes smaller.

Schedule of Events in Fertilized and Unfertilized Eggs

Table II, to which we have already referred, gives the time at which the different events described may be seen by the use of the high powers of the dissecting microscope. The perivitelline space in the unfertilized egg takes from 10 to 30 minutes longer to form than it does in the fertilized egg. There is also less variation in the time of its formation in the fertilized than in the unfertilized egg. Seventy minutes after insemination, fertilized eggs almost regularly develop blastodiscs. Eggs which have not been inseminated take from 90 minutes to an hour and a half to form blastodiscs similar in appearance. A few blastodiscs do not present precisely the same appearance as those which occur normally in fertilized eggs.

The disappearance of the platelets in unfertilized eggs occurs about one hour after stripping and the process itself is much slower from beginning to end than is the corresponding disappearance in fertilized eggs. The slowness of the reaction in unfertilized eggs permits observations which are less practicable in fertilized eggs. Sometimes it is possible to see an unfertilized egg with a small blastodisc and a few platelets and as the platelets disappear, the blastodisc becomes larger. In some eggs, the platelets will disappear and the blastodisc arises from a structureless beginning. In other eggs, the platelets come together in clusters which seem to stream toward the blastodisc. (Plate I, Fig. 2.) They may encircle a blastodisc (Plate I, Fig. 3) which becomes larger as the platelets disappear. A fully developed blastodisc is never found in the presence of platelets in either fertilized or unfertilized eggs. The size of the blastodisc seems inversely proportional to the quantity of platelets present. These facts urge the conclusion that the platelets are directly concerned in the formation of the blastodisc. Ryder (1884) observed the same phenomenon in the eggs of the cod (*Gadus morrhua*) and came to the same conclusion.

A comparison of the behavior of fertilized eggs and of unfertilized eggs placed in sea water shows that the time required for the processes of development is briefer and varies less in the case of the fertilized eggs than in that of the unfertilized eggs, and that these processes occur in a greater percentage of the fertilized eggs. These facts would appear to indicate that these processes are not fully independent of the sperm, but that the sperm in some way hastens the early stages of development of the egg.

The blastodisc in uninseminated eggs occasionally gives the appearance of aberrant cleavages. (See Figs. 6 and 7, Pl. I.) These may be artefacts due to osmotic or to other effects or may be attempts at cleavage which fail because of inadequate stimulus. They resemble

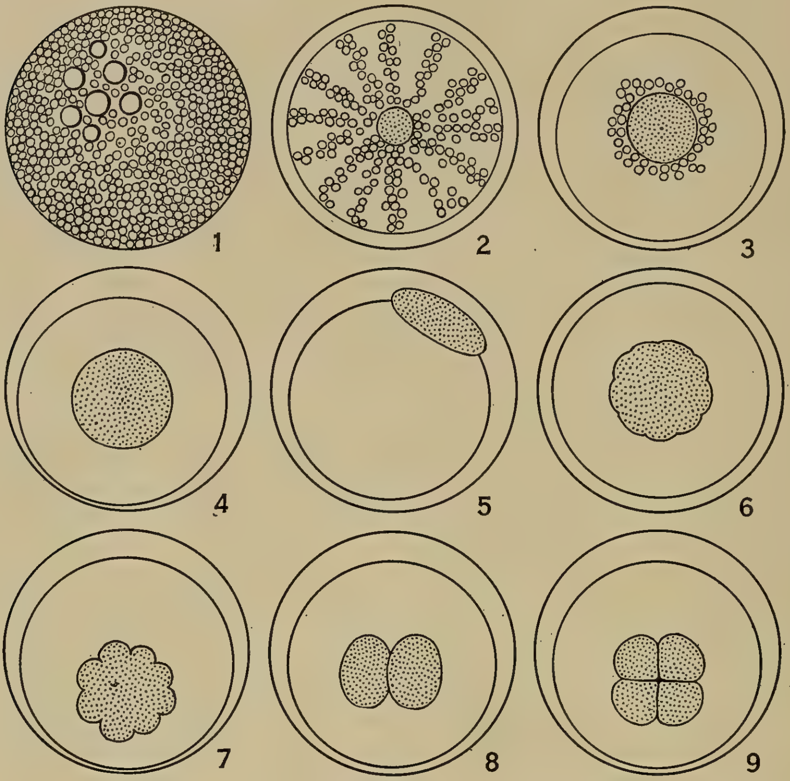


PLATE I

Fig. 1. A freshly stripped unfertilized egg. Note platelets and large oil drops.

Fig. 2. The streaming of the platelets toward a small blastodisc in an unfertilized egg one hour after stripping.

Fig. 3. The same egg as in Fig. 2, but fifteen minutes later. The blastodisc has become larger, and the platelets which are still present surround the blastodisc.

Fig. 4. A normal blastodisc as it appears in a fertilized egg. Most blastodiscs which develop upon unfertilized eggs in sea water present the same appearance.

Fig. 5. Semi-profile view of the blastodisc in an unfertilized egg in sea water. The appearance is identical with the same stage in a fertilized egg.

Figs. 6 and 7. Apparent attempts at cleavage among eggs which have remained in sea water for over three hours. None have ever been seen to cleave completely.

Figs. 8 and 9. Normal two and four-cell stages respectively.

very closely the aberrant cleavages described by Gee (1916) for eggs which were "fertilized" after treatment with alcohol. This was the basis of the suggestion that Gee might have obtained these same aberrant cleavages in alcohol if he did not inseminate his eggs. The season for obtaining *Fundulus* eggs was somewhat shorter during the summer of 1934 than it was in 1933, so that it was not possible to complete experiments planned to determine the truth of this suggestion.

The Effects of Delayed Fertilization upon Cleavage and Hatching

Gee (1916) mentioned that when *Fundulus* eggs are inseminated about a half-hour after stripping, some of the cleavages in the fertilized eggs are abnormal. We have also noted that eggs which are inseminated after 20 minutes in sea water show a high percentage of irregular cleavages. Such eggs have been separated from those which appear normal and another lot which had been properly inseminated at the same time was used as a control. Ninety-five per cent of the control eggs hatched, while only 34 per cent of those which had shown irregular cleavages hatched. Of the latter lot, 71.5 per cent did develop what appeared to be a normal vascular system. The fact that some eggs show an uneven number of cells upon cleavage is suggestive that perhaps the mechanism for the prevention of polyspermy is not as active in aged cells which do not develop a perivitelline space as it is in fresh eggs. Therefore, from the standpoint of normality and the percentage of fertilized and hatching eggs, the investigator is urged to inseminate within 15 minutes after stripping.

Summary

1. Sea water initiates development in the unfertilized eggs of *Fundulus heteroclitus* through the blastodisc stage, including the disappearance of platelets and formation of the perivitelline space. The blastodiscs of such eggs do not cleave. Eggs vary considerably in the rate and susceptibility to activation by sea water.
2. Fresh water and distilled water do not initiate development as efficiently as does sea water.
3. The fertilizable period of the average *Fundulus* egg in sea water is from 15 to 20 minutes.
4. A description of the passage of sperm through the micropyle system is given.
5. The perivitelline space appears to be formed principally by a change in the permeability of the egg cortex. As the yolk shrinks, it

leaves the vitelline membrane to retain the original shape and size of the egg.

6. Evidence is adduced to show that the platelets are directly concerned in the formation of the blastodisc.

7. Although the early stages of development may be initiated in unfertilized eggs by the action of sea water, these stages are not independent in the fertilized eggs. They occur sooner and are of shorter duration in fertilized eggs. Furthermore, the percentage of eggs which develop these stages is higher in the fertilized than in the unfertilized eggs.

8. Insemination which is delayed for twenty or more minutes not only reduces the percentage of fertilization but also increases the percentage of abnormalities and decreases the percentage of hatched eggs in those lots which are fertilized.

LITERATURE CITED

Delage, Y., 1901. Etudes expérimentales sur la maturation cytoplasmique et sur la parthènogenèse artificielle chez les Echinodermes. *Arch. de Zool. Expér. et Gén.,* (Ser. 3) **9**: 285.

Gee, W. P., 1916. Effects of Acute Alcoholization on the Germ Cells of Fundulus heteroclitus. *Biol. Bull.,* **31**: 379.

Just, E. E., 1930. The Present Status of the Fertilizin Theory of Fertilization. *Protoplasma,* **10**: 300.

Kellicott, W. E., 1913. General Embryology.

Kellicott, W. E., 1916. The Effect of Low Temperature upon the Development of Fundulus. *Am. Jour. Anat.,* **20**: 449.

Lillie, F. R., 1916. The History of the Fertilization Problem. *Science,* N.S., **43**: 39.

Loeb, J., 1913. Artificial Parthenogenesis and Fertilization. University of Chicago Press.

Loeb, J., 1915. On the Nature of the Conditions which Determine or Prevent the Entrance of the Spermatozoon into the Egg. *Am. Nat.,* **49**: 257.

Moore, C. R., 1916. On the Superposition of Fertilization on Parthenogenesis. *Biol. Bull.,* **31**: 137.

Moore, C. R., 1917. On the Capacity for Fertilization after the Initiation of Development. *Biol. Bull.,* **33**: 258.

Newman, H. H., 1905. On Some Factors Governing the Permeability of the Egg Membrane by the Sperm. *Biol. Bull.,* **9**: 378.

Newman, H. H., 1907. Spawning Behavior and Sexual Dimorphism in Fundulus heteroclitus and Allied Fish. *Biol. Bull.,* **12**: 314.

Newman, H. H., 1908. The Process of Heredity as Exhibited by the Development of Fundulus Hybrids. *Jour. Exper. Zool.,* **5**: 504.

Nicholas, J. S., 1927. The Application of Experimental Methods to the Study of Developing Fundulus Embryos. *Proc. Nat. Acad. Sci.,* **13**: 695.

Ryder, J. A., 1884. The Embryography of Osseous Fishes with Special Reference to the Development of the Cod (Gadus Morrhua). Washington, D. C.

Stockard, C. R., 1906. The Development of Fundulus heteroclitus in Solutions of Lithium Chlorid, with Appendix on its Development in Fresh Water. *Jour. Exper. Zool.,* **3**: 99.

STOCKARD, C. R., 1907. The Influence of External Factors, Chemical and Physical, on the Development of Fundulus heteroclitus. *Jour. Exper. Zool.,* **4**: 165.

WILSON, E. B., 1903. Experiments on Cleavage and Localization in the Nemertine Egg. *Archiv. f. Entw. Mech.,* **16**: 411.

WOODWARD, A. E., 1918. Studies on the Physiological Significance of Certain Precipitates from the Egg Secretions of Arbacia and Asterias. *Jour. Exper. Zool.,* **26**: 459.

MARINE BIOLOGICAL LABORATORY

SUPPLY DEPARTMENT

WOODS HOLE, MASS.

•

DURING these summer months the Supply Department is stocking new materials, all of which are listed in the new 1935 catalogue now ready for mailing. Request your copy now and it will be mailed immediately.

Our materials are put up by preparators of many years' experience, and they have given satisfaction for nearly half a century.

We would be pleased to have the opportunity of quoting on your needs for summer school and fall classes.

•

Est. 1890

CONTENTS

Page

THIRTY-SEVENTH REPORT OF THE MARINE BIOLOGICAL LABORATORY 1

CLEVELAND, L. R.
The Centrioles of Pseudotrichonympha and their Rôle in Mitosis 46

CLEVELAND, L. R.
The Intranuclear Achromatic Figure of Oxymonas grandis sp. nov 54

YEAGER, J. FRANKLIN AND OSCAR E. TAUBER
On the Hemolymph Cell Counts of Some Marine Invertebrates 66

FRIEDMANN, HERBERT
Notes on Differential Threshold of Reaction to Vitamin D Deficiency in the House Sparrow and the Chick 71

JUDAY, C., AND H. A. SCHOMER
The Utilization of Solar Radiation by Algae at Different Depths in Lakes 75

KILLE, FRANK R.
Regeneration in Thyone briareus Lesueur following Induced Autotomy 82

BACHEM, A., AND M. A. DUSHKIN
A Study of Bacterial Sensitivity to Ultraviolet Radiation 109

DOYLE, W. L., AND C. W. METZ
Structure of the Chromosomes in the Salivary Gland Cells in Sciara (Diptera) 126

STUMP, A. B.
Observations on the Feeding of Difflugia, Pontigulasia and Lesquereusia 136

TURNER, CLARENCE D.
The Effects of Antuitrin-S on the Male Genital Organs of the Lizard (Eumeces laticeps) during Seasonal Atrophy 143

CHASE, H. Y.
The Origin and Nature of the Fertilization Membrane in Various Marine Ova 159

KAGAN, BENJAMIN M.
The Fertilizable Period of the Eggs of Fundulus heteroclitus and Some Associated Phenomena 185

Volume LXIX Number 2

THE
BIOLOGICAL BULLETIN

PUBLISHED BY
THE MARINE BIOLOGICAL LABORATORY

SMITHSONIAN INSTITUTION
OCT 21 1935
NATIONAL MUSEUM

Editorial Board

GARY N. CALKINS, Columbia University
E. G. CONKLIN, Princeton University
E. N. HARVEY, Princeton University
SELIG HECHT, Columbia University
LEIGH HOADLEY, Harvard University
M. H. JACOBS, University of Pennsylvania
H. S. JENNINGS, Johns Hopkins University
E. E. JUST, Howard University
FRANK R. LILLIE, University of Chicago
CARL R. MOORE, University of Chicago
GEORGE T. MOORE, Missouri Botanical Garden
T. H. MORGAN, California Institute of Technology
G. H. PARKER, Harvard University
W. M. WHEELER, Harvard University
EDMUND B. WILSON, Columbia University

ALFRED C. REDFIELD, Harvard University
Managing Editor

OCTOBER, 1935

Printed and Issued by
LANCASTER PRESS, Inc.
PRINCE & LEMON STS.
LANCASTER, PA.

THE BIOLOGICAL BULLETIN

THE BIOLOGICAL BULLETIN is issued six times a year. Single numbers, $1.75. Subscription per volume (3 numbers), $4.50.

Subscriptions and other matter should be addressed to the Biological Bulletin, Prince and Lemon Streets, Lancaster, Pa. Agent for Great Britain: Wheldon & Wesley, Limited, 2, 3 and 4 Arthur Street, New Oxford Street, London, W.C. 2.

Communications relative to manuscripts should be sent to the Managing Editor, Marine Biological Laboratory, Woods Hole, Mass., between June 1 and October 1 and to the Institute of Biology, Divinity Avenue, Cambridge, Mass., during the remainder of the year.

INSTRUCTIONS TO AUTHORS

Preparation of Manuscript. In addition to the text matter, manuscripts should include a running page head of not more than thirty-five letters. Footnotes, tables, and legends for figures should be typed on separate sheets.

Preparation of Figures. The dimensions of the printed page (4⅜ x 7 inches) should be borne in mind in preparing figures for publication. Drawings and photographs, as well as any lettering upon them, should be large enough to remain clear and legible upon reduction to page size. Illustrations should be planned for sufficient reduction to permit legends to be set below them. In so far as possible, explanatory matter should be included in the legends, not lettered on the figures. Statements of magnification should take into account the amount of reduction necessary. Figures will be reproduced as line cuts or halftones. Figures intended for reproduction as line cuts should be drawn in India ink on white paper or blue-lined coördinate paper. Blue ink will not show in reproduction, so that all guide lines, letters, etc. must be in India ink. Figures intended for reproduction as halftone plates should be grouped with as little waste space as possible. Drawings and lettering for plates should be made directly on heavy Bristol board, not pasted on, as the outlines of pasted letters or drawings appear in the reproduction unless removed by an expensive process. Methods of reproduction not regularly employed by the Biological Bulletin will be used only at the author's expense. The originals of illustrations will not be returned except by special request.

Directions for Mailing. Manuscripts and illustrations should be packed flat between stiff cardboards. Large charts and graphs may be rolled and sent in a mailing tube.

Reprints. Authors will be furnished, free of charge, one hundred reprints without covers. Additional copies may be obtained at cost.

Proof. Page proof will be furnished only upon special request. When cross-references are made in the text, the material referred to should be marked clearly on the galley proof in order that the proper page numbers may be supplied.

Entered October 10, 1902, at Lancaster, Pa., as second-class matter under Act of Congress of July 16, 1894.

Vol. LXIX, No. 2 October, 1935

THE

BIOLOGICAL BULLETIN

PUBLISHED BY THE MARINE BIOLOGICAL LABORATORY

AN EXPERIMENTAL ANALYSIS OF THE SEXUAL BEHAVIOR OF THE PRAYING MANTIS (MANTIS RELIGIOSA L.)

K. D. ROEDER

(*From Tufts College, Massachusetts*)

Historical Introduction

The spectral and dignified appearance of the praying mantis has stimulated the imaginations of peasant peoples for several centuries. Owing to the curious praying attitude in which the front pair of legs are held when in repose, the mantis has been associated with a number of semi-religious superstitions, the popular name in France being " prie dieu " or " prega dieu," in Germany " Gottesanbeterin," while the name mantis is derived from the Greek word for prophet or seer.

Disregarding its supposed occult powers, the mantis has some very remarkable habits, and during the last fifty years a number of observations on its life history have been published. Howard (1886) records the cannibalistic habits of *Stagmomantis carolina,* a species indigenous to North America. When a male was placed in the same cage with a female he appeared to be frightened and attempted to escape. The female seized him and commenced to eat the head and thorax. During this process the male showed great sexual excitement, and even minus a head, managed to clamber onto the back of the female and successfully copulate with her. The remains of the male consisting of the thorax and abdomen remained attached to the female for several hours, when it still showed signs of life. Ultimately the male relaxed his grasp on the female, who finished her meal, leaving only the wings and harder pieces of chitin. Riley and Howard (1892) made essentially similar observations on the same species. Fabre (1897) also observed this sexual cannibalism and states that the male may continue to copulate while he is being devoured. In a case mentioned by him one female ate seven males. The observations of Fabre were made upon *Mantis religiosa,* the common European species which has become established in this country, and which is the subject of this paper. Przibram

(1907) describes the life history and habits of *Mantis religiosa,* and discusses the wide variations of coloration in both sexes. Rau and Rau (1913) describe the life history and behavior of *Stagmomantis carolina,* and state that both sexes may mate a large number of times, and that the male may be devoured before he is able to clasp the female, or during or after copulation. Chopard (1914) records the case of a decapitated female *M. religiosa* which deposited a perfectly normal egg mass and lived for a considerable time. Rabaud (1916) confirms the earlier observation of sexual cannibalism, and maintains that the sexual behavior of a decapitated male is exactly similar to that of a normal insect. He also suggests that the cerebral ganglion exerts an inhibitory influence upon the copulatory reflex. Didlake (1926) describes methods for rearing several species of mantis in captivity. Richards (1927) refers to cannibalism among the Mantidæ as described by other writers. Aoki and Takeishi (1927) state that the copulatory act in the male is initiated by destruction of the head. Dubois (1929) states that an artificially decapitated male will copulate with a female, while the latest contribution to the literature is a paper by Binet (1931), who gives an admirable summary of the knowledge of *Mantis religiosa* and adds a number of observations of his own.

A study of the life history and habits of the praying mantis, as described by the writers mentioned above, suggests several interesting questions. All accounts agree as to the ferocity of the female, and her tendency to capture and devour the male at any time, whether it be during the courtship or after copulation. Fabre mentions a case where a number of males were eaten by one female which still remained unfertilized. Such a state of affairs seems hardly to be to the advantage of the species, especially if the slaughter of the male occurs before copulation is achieved, since the sex ratio is about one to one. If the male is eaten during or after copulation he serves the rather doubtful double rôle of fertilizing the female and providing nourishment for the developing eggs, though if he survives he is able to fertilize a number of females (Rau and Rau). However, the writer was unable to discover any account of the behavior of the male during his approach to the female, and it was felt that an examination of this and of the conditions under which successful copulation occurs might furnish an explanation of this curious sexual cannibalism. It must be noted also that several writers record successful copulation after the head and part of the prothorax of the male have been devoured. The reflex mechanism of this curious adaptation has also been investigated during the present study.

The Preliminary Courtship

The insects used in these observations were all reared from the egg in captivity, and while the writer has had no opportunity to observe these insects in the wild state, it is felt that the evidence presented will show that artificial conditions and even the mere presence of an observer contribute to the mortality of the males.

Four successive generations have been raised, and the insects responded readily to captivity at all stages. The only real difficulty experienced was in obtaining a sufficient supply of living insects as food. Practically any insects are eaten provided they are not too large, and they are captured in a very curious manner. The first pair of legs are highly modified as raptorial organs. The femur and tibia are both armed with a double row of strong spines along their posterior borders, and can be flexed on each other so that any object is firmly grasped between these spines. Unless very hungry the mantis remains motionless until an insect moves within reach of the long antennæ. The prey is lightly touched with these, and the raptorial forelegs are extended with lightning rapidity. The femur is flexed on the tibia and the insect firmly held between the spines, while it is torn apart by the powerful mandibles and eaten. (See Fig. 6.) If living insects are scarce or the mantids are crowded they will readily attack and eat one another. Morgue (1909) has even recorded the capture of a small lizard by a large female mantis.

No attempt will be made to give a detailed description of the anatomical differences between the sexes. Reference should be made to Berlese, the Cambridge Natural History, Volume 5, or to any good textbook of entomology. The female averages from 7 to 9 cm. from the head to the tip of the abdomen; the male is somewhat smaller (see Fig. 3). The eyes and antennæ of the male are larger and there is every indication that his visual acuity is greater than that of the female, though both sexes have excellent vision—a condition usually found in rapacious insects. It is of importance to note here that though both sexes will react to the movements of an object no larger than themselves at a distance of a meter or more, they are totally unaware of the presence of any object, however near, provided it does not move. It is curious to observe a mantis stalking a small fly which is making slight movements two feet away, while a motionless fly within reach of the raptorial legs may pass completely unnoticed. If the visual attention of a mantis is attracted by a moving fly which then becomes motionless, the mantis is likewise unable to stalk or grasp the fly unless it makes further movements. In addition to better sensory equipment, the male is slightly more active than the female, and his movements are more rapid and jerky. The female is physically much more powerful than the male, and overcomes him with ease in a struggle. With regard to the genitalia, the ovipositor is rather short and of typical orthopteran type, and is partially enclosed by the upturned edges of the last sternum. (See Fig. 7.) The curious claspers of the male are highly asymmetrical and the broad ninth sternum is twisted to the left about the long axis of the body. (See Fig. 8.) During copulation the tip of the male abdomen is bent round in a pothook-like curve to the left so that the genitalia are directed forward, and also twisted through an angle of 90° so that the last male sternum is to the left side of the female (see Fig. 4.)

For a period of from one to two weeks after the final moult the sexes betray little interest in each other. If they are placed together during this period the female may seize and eat the male as she would any other insect, while the male will show signs of fright at her approach, and frequently makes a rapid retreat. A motionless male is sometimes observed, following every movement of the female with his eyes, but at first no attempt is made to approach her. Towards the end of two weeks the visual attention of the male becomes more and more concentrated on the female. It is out of these periods of concentrated watching on the part of the male that the final courtship develops.

If a male and female mantis which are ready for copulation are placed together in a large well lighted cage, at first they will probably be unaware of each other's presence, and will move about, clean themselves, or catch other insects. Eventually the movements of one will be detected by the other. If the female catches sight of the male first, he will be simply watched or will be approached, grasped with the raptorial legs, and eaten. It normally happens if the cage is large and the pair undisturbed that the male, owing to his better vision, will see the female first. He immediately turns his mobile head in her direction so that she is directly in the line of vision of both his eyes, and then becomes completely motionless or "freezes." The only detectable movement is the reduced vibration of the long antennæ, and this pose may be maintained for several hours. (See Fig. 1.) So extreme is this immobility that if a male is in the act of raising a leg when first the female is detected, it will be kept poised in the air for some time, and many curious positions may be observed.

It can be demonstrated that this curious freezing reaction is initiated in the male purely by the visual image of the female, and not through any other sense. A male will freeze when separated from the female by a glass plate. If the female, though near, passes out of sight, the male soon resumes his normal activities. A blinded male is completely unaware of the proximity of the female, and soon betrays himself by his movements and is caught and eaten. The male may freeze when several feet away from the female, and gentle prodding or teasing which under other circumstances elicits a reaction, will not make the male move or take his eyes off the female. This freezing of the male may possibly be a form of death feigning which is also observed occasionally in the praying mantis.

If the female also remains motionless for a long time the male may lose sight of her, owing to the inability of mantids to detect stationary objects, but under normal circumstances she makes a number of slight movements,—cleans the tarsi and antennæ, catches flies, etc. This apparently stimulates the male to commence a series of very slow almost imperceptible steps in her direction. The progress of the male is best described as slow creeping or stalking, and is quite unlike the normal locomotion. The female is usually approached from behind, but frequently she will detect the slight movements of the male. She reacts by a quick half turn so that both eyes are directed towards him, and then remains motionless. (See Fig. 2.) The male reacts to this sudden movement on the part of the female by promptly freezing, and she soon either loses him, owing to his immobility, or her attention is attracted by the movements of some other insect. It should be noted here that if the male is forced to make further movements while the female is watching him, he may be attacked and eaten.

When the attention of the female is distracted elsewhere the male continues his slow advance, which may be further disturbed by its detection on the part of the female. This hesitant stalking may continue for many hours, and may be ended by the disappearance of the female from the male's line of vision. Typically it ends in the following fashion. The male gets so near to the female that he can touch the tips of her wings with his long antennæ. These are now vibrated more rapidly, and after a further hesitation the male half opens his wings, makes a small jump, and lands on the back of the female. The male clasps her with the raptorial legs, the tibiæ fitting into small grooves on the antero-ventral aspect of the mesothorax, just in front of the bases of the wings. The tarsi of the second and third pairs of legs are placed on the edges of the female's wings and serve further to steady and support the male. The reaction of the female is completely negative once this stage has been reached successfully, and she shows no sign that she recognizes the presence of the male. Likewise she makes no attack on him except under certain circumstances to be described later.

This first phase of the sexual behavior, commencing with visual recognition by the male and ending with the physical contact of the pair will be termed the preliminary courtship. A typical case has just been described, but certain minor variations have been observed. Firstly, the male, though intently watching the female, may make no attempt to approach her. This was seen frequently in immature males (during the first ten days after the final moult). Secondly, the male may remain motionless but the female may chance to move into his proximity so that he can reach her back without stalking her. He then jumps onto her back as described above. Thirdly, the female may approach the male without apparently noticing him, owing to his immobility, thus bringing them face to face. Under these circumstances the male is unable to reach the back of the female in the normal way, and he usually shows great agitation, jumping or flying if possible to some distance. Although this last eventuality occurred only once or twice in the writer's experience, the male moved away and did not attempt to mount the female.

The female has been observed to attack and eat the male if she sees him first. If she is hungry she will then approach him rapidly and grasp and eat him; also, as already mentioned, if the male moves or is forced to move while the female is watching him during his approach. Another insect moving between them or the shadow of the observer will cause the male to lose sight of the immobile female, and possibly result in some movement on his part. If the male approaches the female from the side, landing on her back at right angles, or instead of clasping the mesothorax with his raptorial arms, grips her by the head or only by the tips of the wings, the female then immediately wheels and grabs him.

Provided the male is able to reach the back of the female and clasp her in the normal fashion as described above, the female was never observed to attack him. A discussion of these observations will be deferred until later, and the period of copulation which naturally succeeds the preliminary courtship will now be described.

Copulation

Once the male has come into physical contact with the body of the female, his whole behavior changes, and great activity succeeds the slow stealthy movements described above. The abdomen, which is dorsal and a little to the right of that of the female, is bent round in acute pothook-like curve to the left, so that the claspers are carried round the left side of the female's wings and abdomen, and directed forward toward the ovipositor. The cerci are in active movement, apparently serving to guide the claspers, and the whole abdomen is moved about in an attempt to locate the ovipositor. Many unsuccessful attempts to insert the claspers are made. In one case a male remained on the back of the female for four hours before he was successful in copulating. Owing to the vigorous movements of the abdomen the male occasionally loses his hold, and partially slips off her back. He usually regains his position without mishap, and in spite of the many attempts to copulate and considerable movement of the male, the female shows no sign of recognition. It should be noted that males were never observed to make these copulatory movements—curving of the abdomen, movements of cerci and claspers, etc., unless very close to, or in physical contact with a female. Finally the female makes her only positive sign of sexual recognition by raising the ovipositor, and the claspers of the male are then inserted between it and the curved sternum. Once actual copulation has been effected, the movements of the male are not so considerable, though the abdomen shows intermittent peristaltic contractions. (See Figs. 3 and 4.) The female moves about in a normal fashion and may capture and eat other insects. Handling does not disturb the couple at this stage and they can be removed from the cage and photographed. After a period of four to five hours the claspers of the male are withdrawn, leaving a large white spermatophore between the curved sternum and ovipositor. He then releases his hold on the female and drops from her back to the floor of the cage (the couple usually hang upside down from a branch—see Fig. 5). Directly they separate the female may show considerable interest in the male, following his movements with her eyes. Since the male usually drops some distance from her back, he is not in a position to be attacked.

The male is only occasionally attacked after he has clasped the female, and then only if his position is abnormal. Once the pair are actually in copula the male is never attacked, while if the male remains near the female after they separate, it might be inferred from her reactions that he is once more in danger. No actual attack was observed at this stage, however.

Experiments on the Sexual Reflexes

A number of the writers mentioned above have recorded the fact that a decapitated male is still able to copulate with the female, and that the whole process appears to follow a normal course. Oviposition of a decapitated female has also been recorded. Rabaud suggests that in *Mantis religiosa* the cerebral ganglion may have an inhibitory effect on the copulatory reflexes, while M'Cracken (1907), working with silk-worm moths, states that there is no augmentation of sexual reflexes after removal of the head. The following experiments were performed in order to determine the true relations between the cerebral and subesophageal centers and the sexual reflexes in *Mantis religiosa*.

Adult insects of various ages after the final moult were used, and various parts of the nervous system were removed. The mantis was rendered immobile by wrapping in several turns of cheese cloth, which effectively entangled the legs and obviated the use of an anæsthetic. In order to remove the cerebral ganglion, a small horizontal incision was made in the chitinous head capsule at the bases of the antennæ. A small sharp knife was then introduced and the cerebral ganglia were separated from the subesophageal ganglion by a horizontal cut. In some cases the cerebral ganglia were then removed, but owing to the fact that a larger incision had to be made, bleeding was considerable. Cutting of the commissures and destruction of the brain *in situ* had essentially the same effect as removing the cerebral ganglia, and since it necessitated only a very small incision and resulted in no appreciable hemorrhage, the insects were stronger and more normal in other respects after the operation. Since it was impossible at the time of the operation to ascertain the exact extent of the lesions produced, the heads of the insects were removed at the end of the observation period, hardened in 10 per cent formalin and carefully dissected under a binocular microscope. The removal of the subesophageal ganglion was simpler, and the insect was either simply decapitated or the commissures between the subesophageal and first thoracic ganglia cut in the neck region. The nerve cord was severed at various levels by a similar method, and in all cases the injuries produced were checked by careful dissection. It was found possible to keep decapitated mantids alive and in good condition for three or four days, while, if the subesophageal ganglion was intact and the mouth parts functional, the insects were able to feed and lived indefinitely.

Explanation of Plate I

Fig. 1. The male mantis (below), intently watching the female. She has not noticed him and is engaged in cleaning the tarsi.

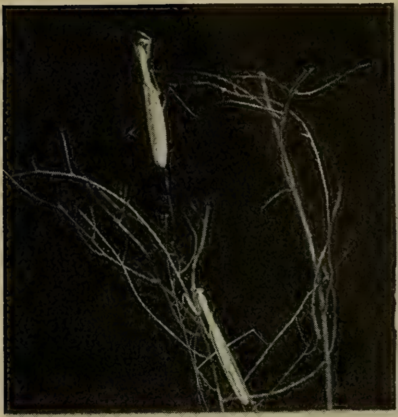
Fig. 2. Taken a few minutes after Fig. 1. The male has moved a little nearer the female. She has detected his movements, and has half turned to face him.

Fig. 3. The same pair in copula. Note how the female is clasped round the prothorax, and the abdomen of the male bent round to the left and rotated through 90°.

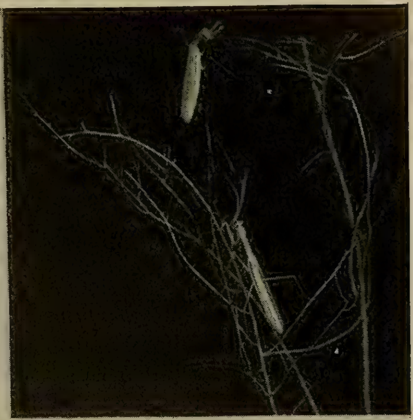
Fig. 4. The same pair seen from below.

Fig. 5. The same pair at the end of copulation. The male has released his hold and removed the claspers, and is about to jump from the back of the female.

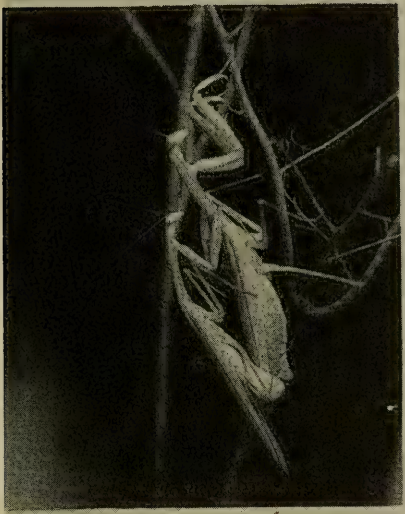
Fig. 6. A female mantis eating another female. The prey has been consumed with the exception of part of the abdomen and one leg. Unfortunately, no good photographs of an attack on a male were obtained.



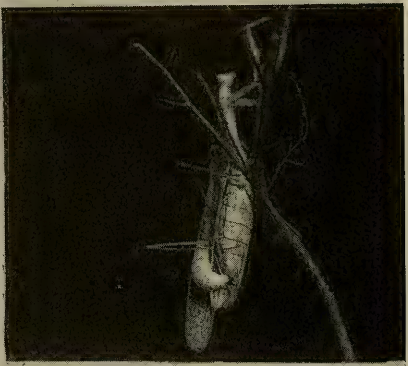
1



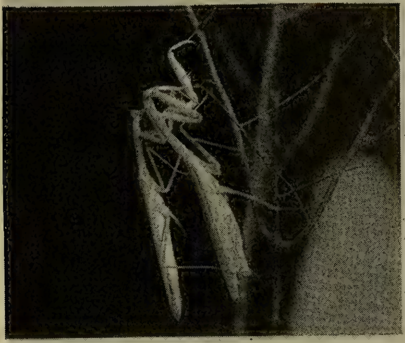
2



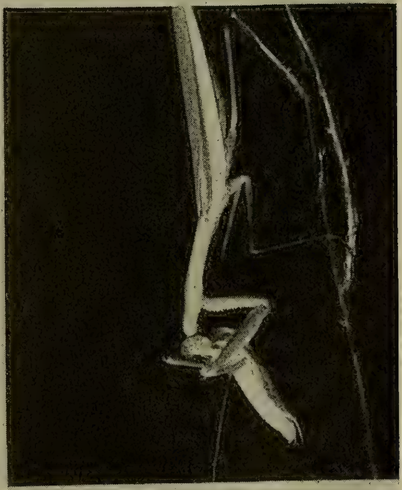
3



4



5



6

Plate I

Removal of the Cerebral Ganglion

This was done in eight males and seven females at various times after the final moult. This operation always results in greatly increased locomotor activity, which will be dealt with in a subsequent paper. But in neither sex at any age has sexual activity been observed. The males show no recognition of the presence of the female, and, owing to their unusual activity, they are invariably attacked. This complete lack of awareness of the proximity of the female is also seen in blinded males, and is undoubtedly due to the lack of the visual and cerebral ganglia. Even if a decerebrate male is placed on the back of a female, he does not grasp her nor show copulatory movements, and owing to the tendency towards continuous locomotor activity soon moves away. The copulatory movements described in normal males were never seen at any time. The female likewise shows no sexual activity.

Removal of the Subesophageal Ganglion

Eighteen males and eighteen females were either decapitated or the ventral nerve cord cut as described above.

Before the reactions of decapitated males are described, it must be repeated that normal males never exhibit copulatory movements except in the close proximity of a female, and then only if they have reached full maturity (ten days after the final moult). After removal of the subesophageal ganglion there is a preliminary stage of shock, during which a variety of movements are observed. This period of shock is essentially similar in both sexes. It is of short duration, averaging about 10 minutes, and the nature of the spontaneous movements observed varies much in different individuals. Finally the insect becomes more or less motionless in a normal standing position, though the legs are more spread and the body nearer the ground owing to a general drop in muscle tone. In the case of the male, slight movements of the claspers, cerci, and abdomen then commence, and gradually increase in intensity until the abdomen is continually bent round in the typical pothook-shaped curve, and the cerci and claspers moved in a manner identical with the normal copulatory movements. These typical copulatory movements are continuous, and are accompanied by very curious locomotor activity which at first could not be accounted for. The male begins to rotate slowly in an arc. The first pair of legs are not moved and the tarsi of these serve as an axis about which the body rotates, the tip of the abdomen moving on the circumference of the arc (see Figs. 9 and 10). This rotation is effected by lateral extensions of the third and to a lesser extent the second leg on one side of the body. The tarsi of these legs grip at the full extension, and as they are flexed the body is drawn sideways. The second and third leg on the other side of the body assist this lateral movement by pushing the body on the extension, being then lifted and flexed passively. Since the third pair of legs are most effective and the first pair are not moved but serve as an anchor, the insect rotates in an arc as described above. This lateral locomotion is accompanied by the copulatory movements of the abdomen, and both continue without a halt for from two to twenty-four hours, being observed for a longer period in older males. It should be noted that both copulatory and lateral locomotor movements were observed in freshly moulted males, which normally exhibit no sexual reactions. Faint copulatory movements of the abdomen were seen even in a male before the final moult. This insect was accidentally eaten by another mantis and showed definite though slight bending of the abdomen to the left. (See Fig. 11.)

Out of eighteen cases, sixteen males showed continuous copulatory movements. Two males showing no copulatory movements died soon after the operation and were probably in bad condition when operated on. Fifteen males showed spontaneous locomotor movements. In four of these the movement was sideways, in five the movement resulted in an anticlockwise rotation as described above, in five there was a clockwise rotation, while one insect moved backwards. In the sixteen cases the locomotor and copulatory movements commenced simultaneously from five to twenty-five minutes after operation. In the ten cases showing rotation, a complete rotation occupied from two to fifteen minutes. Although in every case the abdomen was bent in a curve to the left, it will be seen that there was considerable variation in the locomotor movements. The difference between the sideways and rotary movements is slight, for they are both produced by lateral extensions and flexions of the second and third pairs of legs. The movement is accomplished by four legs since the first pair are motionless, and if the movements of the second pair are equal in magnitude to those of the third pair and the tarsi of the first pair do not catch in some object, a lateral crab-like movement in a straight line results. If an insect moves to the right it will always move in that direction, and vice versa. It is not clear, however, why some insects move anticlockwise (to the left) and others clockwise (to the right), while in all cases the abdomen is bent in the same direction—to the left.

These sideways movements are seen only while the males are on a plane surface such as a table top. If they encounter any rounded object such as a pencil or the observer's finger, it is immediately grasped by the forelegs, while the other legs steady the body. Violent attempts are made to copulate with the object, and all attempts at locomotion cease. If a rotating male is carefully placed on the back of a female, she is immediately clasped in a similar fashion, and attempts are made to insert the claspers between the ovipositor and sternum (see Fig. 12). The female makes no attempt to dislodge or attack the decapitated male, his advances are almost immediately accepted, and a normal copulation ensues resulting in the formation of a spermatophore.

Removal of the subesophageal ganglion in the female results in less astonishing but comparable reactions. Eighteen females were treated in this fashion. Locomotor movements which are so apparent in similarly treated males are never observed in females, which if undisturbed remain absolutely stationary until death. The abdomen, however, shows signs of muscular activity. The tip is pressed down against the substratum, the cerci moved, and the ovipositor makes slight up and down movements similar to those seen during the construction of the oötheca by a normal female. These movements of the abdomen and ovipositor are slight, but distinctly seen in females of all ages, and never occur in unoperated females, except for a few minutes prior to oviposition. Unfortunately none of the females experimented with contained fully ripe eggs, consequently no actual oviposition occurred. It is therefore not possible to say whether normal oötheca formation can occur in the absence of the subesophageal ganglion. This has, however, been recorded by Chopard.

A decapitated female will readily accept a male, decapitated or otherwise, and actual copula results sooner than when both insects are intact. The pair remain together for about four hours, and a normal spermatophore is deposited (see Fig. 13).

Explanation of Plate II

Fig. 7. The abdomen of a preserved female. It is much broader and shorter than that of the male.

Fig. 8. The abdomen of a preserved male (the wings have been clipped). The abdomen is held in this position in normal males except when clasping a female. The asymmetrical claspers can also be seen.

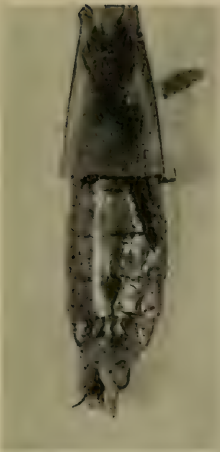
Fig. 9. A decapitated male. The bending and rotation of the last few abdominal segments can be seen. This insect was rotating about the first pair of legs in an anticlockwise direction (to the left).

Fig. 10. A decapitated male, showing behavior similar to the last. The wings were clipped to show the curve in the abdomen.

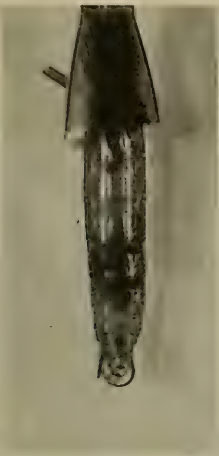
Fig. 11. A very immature male. The head and prothorax were removed before the final moult (note the immature wing pads). The abdomen shows slight bending characteristic of the copulatory movements.

Fig. 12. A decapitated male clasping a normal female. The abdomen is strongly bent in an attempt to insert the claspers. This attempt was subsequently successful.

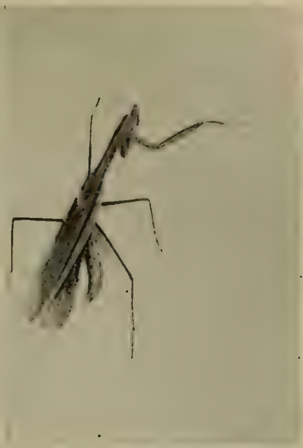
Fig. 13. A decapitated male in copula with a decapitated female (compare with Fig. 4). Both insects were decapitated before they were placed together.



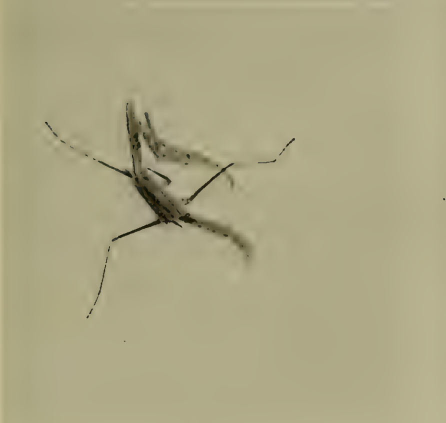
7



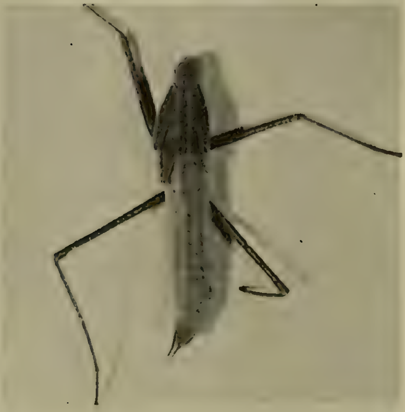
8



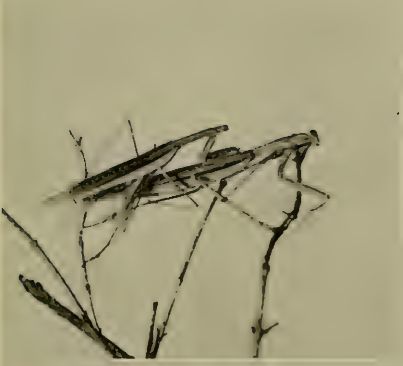
9



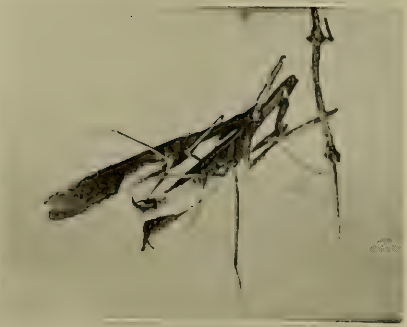
10



11



12



13

Plate II

Separation of the Thoracic and Abdominal Ganglia

After decapitation a male mantis shows (1) continuous lateral movements, (2) continuous copulatory movements. If the abdomen and thorax are now separated, copulatory movements of the same magnitude always occur in the isolated abdomen. If the nerve cord in the abdomen is now cut at various levels, the movements of the cerci and claspers continue while the last abdominal ganglion is intact. The muscular movements which result in the twisting of the abdomen are reduced or absent if the muscles concerned are separated from the last abdominal ganglion. These results have been obtained in every case. The effects of separation of the thorax and abdomen on the lateral locomotor movements are not so clear cut. Owing to the disturbance in weight distribution on the loss of the abdomen, the insects are usually unable to remain on their feet. If, in order to counteract this, the nerve cord only is separated, leaving the abdomen and thorax attached, spontaneous attempts to walk occur in about half the insects. The locomotor movements may produce a lateral or sometimes a backward progression, or there may simply be random movements of the legs. It is interesting to note that where locomotion occurs in a nervously isolated thorax, it does not continue for very long after the operation, lasting usually for from five minutes to half an hour.

In the female the typical and continuous ovipositor movements seen in decapitated specimens persist in the isolated abdomen and continue while the last abdominal ganglion is intact.

Discussion and Conclusions

From the foregoing account it will be realized that the female mantis is extremely voracious, and frequently attacks and eats the male in addition to other insects. However, the sexual behavior of the male, which tends to increase his chances of successful copulation, can be divided into two distinct phases, (1) the preliminary courtship, and (2) the copulation.

Preliminary Courtship

This phase of the sexual behavior begins the moment the male sees the female, and ends when he reaches the mating position on her back. It may or may not (in immature males) be followed by copulation, but is its normal precursor. The whole behavior of the male during the preliminary courtship is dominated by the sense of vision, and he becomes aware of the female and approaches her only when he is able to see her. The preliminary courtship in the male is characterized by long periods of complete immobility alternating with very slow movements in the direction of the female. Since decerebrate insects of both sexes are almost continually in movement but show no sexual reflexes, there must be centers in the cerebral ganglion which impose a partial inhibition on the locomotor centers in the ventral nerve cord. The immobility of the male when aware of the female could on this basis be interpreted as being due to stimulation of these inhibitory centers by impulses coming from the optic ganglia.

This immobility and slow stalking offer a very real protection to the male as he approaches the female, and greatly increase his chances of successful copulation. This is shown by the fact that the male is frequently attacked and eaten if (1) the female sees him first, (2) if her attention is attracted by artificially induced movements on his part, or (3) if his line of vision is temporarily interrupted during his approach. The large number of cases of sexual cannibalism which have been reported in this species may be due to one of these eventualities arising either from the confined quarters of the pair or from disturbing movements on the part of the observer.

The male therefore wins the female not by display but by the absence of display. If he is able to reach her unobserved and make his final jump onto her back, he is not molested. He may, however, approach the female undetected by her, only to clasp her in some abnormal position and be attacked and eaten. Discussed below is a very beautiful reflex mechanism which takes care of this eventuality.

It is debatable whether there is a similar period of preliminary courtship in the sexual behavior of the female. Her actions during the approach of the male seem to indicate that he is regarded as any other insect, and consequently suitable as food. Her cannibalistic tendencies must, however, act in a selective fashion, promoting absence of display in the male.

Copulation

This phase of sexual behavior commences in both sexes at physical contact and ends when they separate. Normally it is preceded by the preliminary courtship. Decapitated males will, however, copulate without any preliminary stalking. The male characteristically clasps the female round the mesothorax with his raptorial legs, while his abdomen shows active copulatory movements that culminate with the insertion of the claspers.

The nerve center responsible for these copulatory reflexes is situated in the last abdominal ganglion. This is demonstrated by their absence after the destruction of this ganglion. In the subesophageal ganglion is a center which is antagonistic to the last abdominal center, inhibiting copulatory movements except when the male is in contact with a female. This is shown by the fact that copulatory movements never occur in intact males of any age, except when clasping a female, while in males minus the subesophageal ganglion there are continuous copulatory movements, attempts to clasp and copulate with any object, and immediate and successful copulation if they are allowed to clasp a female. The question arises as to what is the mechanism which prevents copulatory movements in intact males unless they are mature and clasping a female. No definite proof has been obtained on this point, but it seems probable that the tactile stimulus occasioned by contact with the body of the female neutralizes or antagonizes the inhibitory center in the subesophageal, thereby releasing the copulatory movements dependent on the last abdominal ganglion.

A little more difficult of interpretation are the curious lateral and rotary locomotor movements seen in males minus the subesophageal ganglion. Evidently they are closely connected with the sexual behavior since they are concurrent with the copulatory movements, and cease when a female or any round object is clasped. After the abdomen is separated from the thorax they continue for a short while and then cease, indicating that they are not so dependent on the last abdominal ganglion as are the copulatory movements.

Taken together, the continuous copulatory and lateral locomotor movements constitute a very beautiful means for securing fertilization of the female, should the preliminary courtship be unsuccessful and the male captured. When the male is attacked by the female he is usually seized by the raptorial arms or the head, and these are devoured first. This results in the destruction of the subesophageal ganglion and the consequent removal of the center inhibiting the copulatory movements. Since the female is grasping the head of the male, the bodies of the two insects are connected at the front end but are free posteriorly. Lateral or rotary movement on the part of the male tends to swing him round until the axis of his body is parallel to and beside that of the female. If the female is not clasping him too far back a continuation of this movement brings him onto the back of the female, and the continuous copulatory movements result in the insertion of the claspers. Thus it has been possible to analyze the sexual reactions of a decapitated male mantis, first described by Howard in 1886.

In the female also, the reflex movements associated with sex originate in the last abdominal ganglion, since isolated abdomens show continuous ovipositor movements. Under normal circumstances these ovipositor movements are inhibited by a center in the subesophageal ganglion as shown by their appearance in unripe females after decapitation. The stimulus which eliminates the inhibitory effect in an intact female at the time of oviposition was not investigated, but is probably internal and associated with the presence in the abdomen of ripe eggs.

SUMMARY

1. A number of writers have reported the capture and eating of the male mantis by the female, both before and during copulation. This sexual cannibalism is promoted by captivity or disturbance of the couple before copulation, and is counteracted by the preliminary courtship of the male.

2. The male has better vision than the female, and detects and approaches her only when she is in sight. His approach is very slow and hesitant, and there is complete absence of any display. If he is able to reach the back of the female undetected by her, he is not attacked.

3. If his movements during the approach attract the attention of the female, or she is clasped in an abnormal position, he is attacked and eaten. Since his head is attacked first, an inhibitory center in the subesophageal ganglion is soon destroyed. This center normally inhibits (1) lateral locomotor movements, (2) copulatory movements of the abdomen, which originate in the last abdominal ganglion.

4. These movements consequently commence after destruction of the head and bring the body of the male into the mating position on the back of the female, and copulation is immediately effected.

5. Ovipository movements in the female are similarly promoted by the last abdominal ganglion, and normally inhibited by the subesophageal ganglion.

BIBLIOGRAPHY

Aoki, B., and S. Takeishi, 1927. On the Copulatory Behavior of the Japanese Mantis (Tenodera aridifolia Stoll.), and the Seat of its Nerve Center. *Dobuts Zarski,* **39**: 114.

Binet, L., 1931. La vie de la Mante religieuse. Vigot Freres, Paris.

Chopard, L., 1914. Sur la vitalité de Mantis religiosa L.; ponte après décapitation. *Bull. Soc. Ent. France,* No. 19, p. 481.

Didlake, M., 1926. Observations on the Life Histories of Two Species of Praying Mantis. *Ent. News,* **27**: 169.

Dubois, R., 1929. See bibliography of L. Binet.

Fabre, J. H., 1897. Souvenirs Entomologiques, Vol. 5.

Howard, L. O., 1886. The Excessive Voracity of the Female Mantis. *Science,* **8**: 326.

M'Cracken, M., 1907. The Egg-laying Apparatus in the Silk Worm (Bombyx mori) as a Reflex Apparatus. *Jour. Comp. Neur.,* **17**: 262.

Morgue, M., 1909. Un reptile chassé et tué par un insecte. Feuille des jeunes naturalistes, p. 87.

Przibram, H., 1907. Die Lebensgeschichte der Gottesanbeterinnen (Fangheuschrecken). *Zeitschr. f. wiss. Insektenbiol.,* **3**: 117–122, 147–153.

Rabaud, E., 1916. Accouplement d'un mâle décapité de Mantis religiosa L. *Bull. Soc. Ent. de France,* No. 1, p. 57.

Rau, P., and N. Rau, 1913. The Biology of Stagmomantis carolina. *Trans. Acad. Sci. St. Louis,* **22**: 1.

Richards, O. W., 1927. Sexual Selection and Allied Problems in the Insects. *Biol. Rev.,* **2**: 298.

Riley, C. V., and L. O. Howard, 1892. The Female Rear-horse Versus the Male. *Insect Life,* **5**: 145.

METHODS FOR THE DETERMINATION OF NITROGEN AND CARBON IN SMALL AMOUNTS OF PLANKTON

THEODOR v. BRAND

(*From the Laboratory of Zoöphysiology, Copenhagen University*)

The amount of plankton in a sample of sea water is very small in comparison with the dissolved organic matter. It is impossible to determine, for example, the nitrogen content of the plankton by subtraction of the nitrogen content in a certain amount of sea water after filtering off the organisms from that found in an analysis of the same water without filtration. It is necessary to concentrate the plankton and make direct determinations. The concentration cannot be carried out by filtration or simple centrifugation but it is necessary to use a combined precipitation and centrifugation method as worked out by Steemann-Nielsen and v. Brand (1934), and further adapted to our special requirements.

We give as an example the description of the concentration of the plankton from 50 ml. sea water for the purpose of a nitrogen analysis. The water is first pipetted off in a suitable centrifuge tube. After the addition of 1 ml. of 2 per cent KOH, a precipitation of hydroxides results, which encloses and carries down all plankton organisms. The precipitate is thoroughly stirred up with a glass rod and centrifuged for 10 minutes at about 3,400 revolutions per minute. After decantation of the supernatant fluid, the precipitate is dissolved in a suitable amount of 3 per cent H_2SO_4 (made up with ammonia-free distilled water). The solution is transferred quantitatively to a smaller centrifuge tube of about 25 ml. and brought up to about 20 ml. by addition of ammonia-free distilled water. To this faintly acid solution are now added as many drops of 2 per cent KOH as are required to produce a just visible turbidity. The material is again centrifuged for about 10 minutes. The fluid above the precipitate is transferred to another centrifuge tube and by addition of a suitable amount of 2 per cent KOH and centrifugation a control precipitate of about the same size is obtained. This procedure is necessary, since we have found that a precipitate of hydroxides in sea water always contains (absorption?) a certain amount of nitrogen and carbon, which cannot be neglected. Therefore, we have not only to make a determination on the precipitate containing the organisms, but also a blank on the control precipitate. It is advisable to make both precipitates as small as possible and as nearly as possible the same size (about 0.1 ml.). To each of both tubes are now added 1.9 ml. of ammonia-free distilled water. The total amount of fluid is then always very nearly 2 ml. The precipitates are thoroughly mixed with the water by means of a glass rod, whereupon the substances are ready for the ignition.

The first steps in the preparation of the material for a carbon determination are the same as described above for the nitrogen determination. After the last centrifugation no water is added, but the precipitate is dissolved with several drops of 3 per cent H_2SO_4. The reaction has to be definitely acid (by litmus). The centrifuge tube plus the dissolved precipitate are weighed with an accuracy of 1 mg., the solution pipetted into another small glass tube, and the centrifuge tube weighed again, so that now the weight (gram = ml.) of the dissolved precipitate is known (generally between 0.25 and 0.45 gram). The control precipitate is treated in the same way. It is unnecessary to weigh it, as the same amounts are always burnt as in the analyses containing the organisms.

The above procedures are valid for analyses of fresh material and also for nitrogen determinations on material preserved with formalin (5 ml. formaldehyde, 20 per cent to 200 ml. sea water). For carbon analyses on preserved material the formaldehyde has to be removed with the greatest care. After the first centrifugation and decantation of the fluid, we dissolve the precipitate, fill up with about 80 ml. of distilled water, precipitate with about 1.5 ml. of 2 per cent KOH and centrifuge again. These manipulations have to be repeated a total of three times, then the fourth precipitate is ready for the ignition. We advise using for the preservation of the plankton the best formaldehyde solution available. We have used with good success formaldehyde Schering. It must be pointed out that the use of formaldehyde can in certain cases seriously interfere with the carbon determination. It is known that sometimes the practically water-insoluble paraformaldehyde is formed in formaldehyde solutions. It is not necessary to insist upon the fact that in such a case a carbon determination would be impossible. We have occasionally found in some analyses of preserved diatom cultures very improbable amounts of carbon. As these cultures had not been preserved for our special purposes, we cannot decide whether the formation of paraformaldehyde or impurities contained in the bottles are responsible for this failure.

As the amounts of nitrogen and carbon in the single analysis are very small, it is necessary to avoid as far as possible all contamination of the water. All glass objects, such as centrifuge tubes, pipettes, etc., must be cleaned with the greatest care with distilled water before use, but it is not advisable to wipe them dry with a cloth. We close the centrifuge tube with tinfoil to avoid contamination with dust during the centrifugation.

Nitrogen-Determination

The analyses are performed according to Krogh and Keys' (1934) method for the determination of organic nitrogen in sea water. As the method has been fully described by Krogh and Keys, it is unnecessary to repeat all details here. We record only the principle of the method and call attention to some points in which our procedure differs from the original method, owing to the different material used.

The material to be analyzed is introduced by means of a pipette into a silver tube and burned (about 500°) with alkali in absence of oxygen. For this purpose a stream of NH_3 and O_2-free hydrogen passes during the whole heating process through the system. During the ignition the organic nitrogen is transformed into ammonia. This is taken up in n/100 HBr and determined according to the method of Teorell (1932) in the modification of Krogh (1934).

Krogh and Keys run into the silver tube through the inlet a water sample, usually of 5 ml., boil off the water, and heat to a dull red heat. As in analyses with sea water some ammonia is included in the salts, they run in afterwards 2 ml. of water poor in nitrogen and evaporate this also. We burn in the single analysis only 1 ml. of the material, so that according to the prescription given above duplicate analyses of each sample can always be performed. Because of the relatively small sample, it is necessary to wash out the glass parts of the inlet. We use for this purpose 1 ml. of water poor in nitrogen. On the other hand, our samples contain much less of salts than sea water. Therefore it is unnecessary to run into the tube 2 ml. of water after the ignition. It is possible for the same reason to perform a whole series of about 8–10 analyses without washing the silver tube.

As shown in Table I, we have made a series of analyses on substances with known nitrogen content. The mean error is about 10 per cent in the range of 1–2γ nitrogen. The only substance which gave absolutely faulty results was pyridin, probably because of its relatively great volatility. It is necessary to observe the following points in such analyses: (1) It is advisable to perform duplicate analyses. (2) All solutions to be burned (*a*) solution with the substance of unknown nitrogen content; (*b*) solution with known amount of ammonium sulphate must be made up with the same redistilled water. In respect to the ammonium sulphate solution, it is sufficient

Table I

Substance in γ		Nitrogen content in γ		Remarks
		Calculated	Found	
Aspartic acid	9.8	1.03	0.98	
Aspartic acid	9.8	1.03	1.02	
Creatinine alum	8.7	1.16	1.14	
Creatinine alum	8.7	1.16	1.23	
Pyridine chloride	13.7	1.05	0.12	
Urea	2.36	1.10	1.22	
Alanine	6.7	1.05	1.08	
Peptone		1.04	1.28	Nitrogen content calculated from macro Kjeldal analyses
		0.98	0.79	Nitrogen content calculated from macro Kjeldal analyses
		0.98	0.74	Nitrogen content calculated from macro Kjeldal analyses
Urine		1.08	1.06	Nitrogen content calculated from macro Kjeldal analyses
		1.10	0.89	Nitrogen content calculated from macro Kjeldal analyses
Yeast*	171	4.0	5.60	Nitrogen content calculated from macro Kjeldal analyses, precipitated
Yeast	76	1.8	1.60	Nitrogen content calculated from macro Kjeldal analyses, precipitated
Yeast	76	1.8	1.80	Nitrogen content calculated from macro Kjeldal analyses, precipitated
Yeast	87	2.0	2.40	Nitrogen content calculated from macro Kjeldal analyses, precipitated
Yeast	87	2.0	2.60	Nitrogen content calculated from macro Kjeldal analyses, precipitated

TABLE I (*Continued*)

Number of organisms in the single determination	Nitrogen content in γ		Remarks
	Found	Calculated for 100,000 organisms	
76,000 Skeletonema costatum	0.58	0.76	Fresh culture, precipitated
152,000 Skeletonema costatum	1.52	1.00	Fresh culture, precipitated
252,000 Skeletonema costatum	2.00	0.79	Preserved with formaldehyde, precipitated
252,000 Skeletonema costatum	2.06	0.82	Preserved with formaldehyde, precipitated
19,200 Chætoceros lacimosus	2.60	13.50	Fresh culture, precipitated

Plankton samples from Iceland (preserved with formaldehyde)		Organisms in 100 ml.	Ml. centrifugated	γN found	γN in 100 ml.
No.	Depth in m.				
4,415	0	503,000	50	3.0	6.0
4,415	0	503,000	50	2.4	4.8
4,415	25	227,000	50	3.8	7.6
4,415	25	227,000	50	3.6	7.2
4,415	25	227,000	20	1.2	6.0
4,412	25	122,000	50	1.8	3.6
4,412	25	122,000	50	1.9	3.8
4,412	10	154,000	50	1.4	2.8
4,412	10	154,000	50	1.0	2.0
4,114	10	275,000	50	1.6	3.2
4,114	10	275,000	50	2.2	4.4
4,433	25	11,000	200	2.0	1.0
4,459	0	33,000	200	3.6	1.8

* Yeast precipitated = yeast suspended in sea water (free of other organisms) and treated like plankton.

when only the final dilution from the stock solution is made up with the water used for the solution of the unknown substance. We use water, which contains as little nitrogen as possible. The water is prepared by distillation first from an alkaline solution of permanganate and again from H_2SO_4 and permanganate. As Krogh and Keys have pointed out, this water still contains some nitrogen. Therefore it is necessary to determine the nitrogen content of the water used and to correct the value obtained in the analyses by subtraction of the nitrogen value of 2 ml. water from that obtained in the sample burned. It is, of course, always necessary to determine the nitrogen value of the water when water from a new distillation is used in the analyses. Beyond that we perform about weekly a new determination of the nitrogen content of the water used. (3) It is advisable always to burn the same amount of water in both series of analyses (water containing the samples + water for washing out the inlet of the silver tube) as this is the only way to avoid errors due to the nitrogen content of the distilled water used. (4) For this same reason, the nitrogen content of the unknown substance should not differ too much from that of the ammonium sulphate solution used.

Examples

1. Nitrogen analysis of 9.8γ aspartic acid = 1.03γ nitrogen dissolved in 1 ml. of water. One ml. wash water.

Titrations of 1 ml. HBr directly:	1.80
	1.82
	1.81
	1.81 ml. naphthyl red
Correction for 2 ml. water:	−0.18
	1.63 ml. naphthyl red
Titrations of 1 ml. burned ammonium sulphate solution containing 1γ nitrogen per 1 ml.; 1 ml. wash water:	1.12
	1.03
	1.10
	1.08 ml. naphthyl red
Directly titrated, corrected:	1.63
1γ ammonium sulphate N:	−1.08
	0.55 ml. naphthyl red corresponding to 1γ nitrogen
Titrations of 1 ml. burned aspartic acid solution; 1 ml. wash water:	1.10
	1.07
	1.05
	1.07 ml. naphthyl red

Directly titrated, corrected:	1.63
Burned aspartic acid solution:	−1.07
	0.56 ml. naphthyl red for the burned aspartic acid

0.55 ml. naphthyl red = 1γ N; 0.56 ml. naphthyl red = 1.02 γ N.
Calculated: 1.03 γ N.
Found: 1.02 γ N.

2. Nitrogen determination in a culture of *Skeletonema costatum*, preserved with formaldehyde. Total number of diatoms: 378,000. Last precipitate diluted to 3 ml.

Titrations of 1 ml. HBr directly:	1.65 1.65
	1.65 ml. naphthyl red
Correction for 2 ml. water:	−0.30
	1.35 ml. naphthyl red
Titrations of 1 ml. burned ammonium sulphate solution containing 1γ N per ml., 1 ml. wash water:	0.69 0.69
	0.69 ml. naphthyl red
Directly titrated, corrected:	1.35
1γ ammonium sulphate N:	−0.69
	0.66 ml. naphthyl red corresponding to 1γ N
Titrations of 1 ml. burned organism precipitate, 1 ml. wash water:	0.64 0.51
	0.58 ml. naphthyl red
Directly titrated, corrected:	1.35
Burned organism precipitate:	−0.58
	0.77 ml. naphthyl red corresponding to 1 ml. precipitate 2.31 ml. naphthyl red correspond to the total precipitate
Titrations of 1 ml. burned control precipitate, 1 ml. wash water:	1.18 1.32
	1.25 ml. naphthyl red
Directly titrated, corrected:	1.35
Burned control precipitate:	−1.25
	0.10 ml. naphthyl red corresponding to 0.1 ml. precipitate 0.30 ml. naphthyl red corresponding to the total precipitate

Total organism precipitate:	2.31
Total control precipitate:	−0.30
	2.01 ml. naphthyl red correspond- to the organisms

0.66 ml. naphthyl red = 1γ N; 2.01 ml. naphthyl red = 3.0γ N.
Nitrogen content of 378,000 *Skeletonema costatum* is 3.0γ N.

With the solutions recommended by Krogh (1934), it is possible to determine ammonia amounts from about 0.5 to 2.5γ nitrogen content. By using about twice as strong solutions, it is possible to determine amounts of nitrogen to 5γ in the single determination. In determinations of plankton with an unknown nitrogen amount, a certain drawback of the method is that, as shown by Teorell (1932), there is no indication whether the nitrogen amount was too high for the hypobromite solution used. It will therefore be advisable in an investigation of the composition of an unknown plankton to perform first a carbon analysis, as the limits for the carbon determination are not so narrow. It will then be possible to calculate the suitable amount of water for the centrifugation, as probably also in plankton the approximate ratio C : N will be from 10 : 1 to 7 : 1.

Carbon-Determination

The principle of the method is the same as used by Krogh (1930) in his method for the determination of dissolved matter in fresh water: The material is burned in a quartz tube with copper oxide as contact substance and the CO_2 formed is determined. As we have to deal with much smaller quantities than in the original method, we have had to work out the modification for this special purpose.

Apparatus needed (Fig. 1).

1. Oxygen cylinder fitted with
2. A needle valve, which can be accurately regulated.
3. Wash-bottle containing 40 per cent KOH.
4. Connecting piece fitted with a cotton plug.
5. Quartz tube of about 25 cm. length and 4 mm. diameter. It is charged on a length of about 10 cm. with copper oxide and fitted at one end of this with an asbestos plug. The ends of the quartz tube must be perfectly smooth.
6. Connecting piece fitted with two cotton plugs.
7. Absorber.
8. Burner.
9. Syringe pipette adjusted to 0.1 ml.
10. Syringe pipette adjusted to 0.4–0.5 ml.

11. Barium hydroxide solution, about n/12 to n/15, prepared and stored according to Krogh and Keys (1934).
12. n/3 HCl.
13. Rehberg burette.
14. Drying apparatus according to Krogh (1930, page 255, Fig. 3), but without the filling arrangement. Instead of this, one end of the quartz tube is connected with a rubber tube fitted with a capillary tube and a dense asbestos plug which will retain all dust particles, while the air is sucked through the tube.

An analysis is performed as follows (Fig. 1): The liquid to be analyzed is introduced into the quartz tube (5) by means of the syringe pipette (9). The quartz tube is then dried in the drying apparatus. We begin by heating the water jacket to about 70° C. and thereupon suck the air through the apparatus. In 5 minutes the quartz tube is dry. It is removed from the drying apparatus and set up by means of the connecting pieces (4 and 6). The system is washed out with oxygen. The oxygen stream is then reduced to about 5 ml./min., the baryta solution introduced into the absorber (7) by means of the syringe pipette (10). Then the two flames at each end of the burner (8) are lighted and only when the quartz tube is at a red heat at those places, the other flames are lighted one by one. The quartz tube has to be heated to a red heat for 5 minutes. After the combustion the absorber is removed and the CO$_2$ titrated according to Krogh and Rehberg (1929, 1930). One mm.^{3} n/3 HCl corresponds to 2γ C.

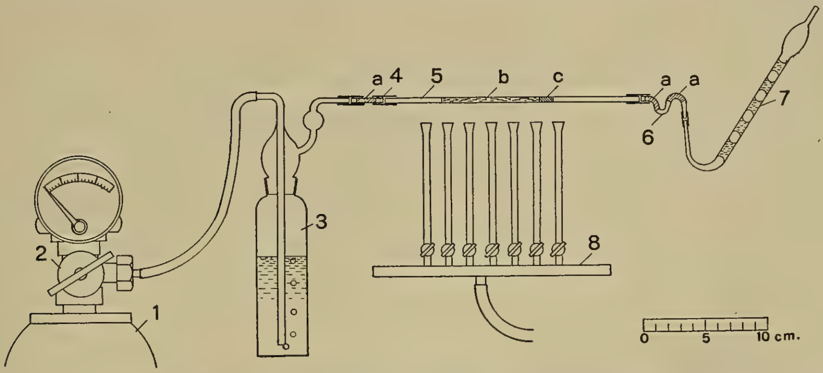


FIG. 1. 1. Oxygen cylinder; 2. Needle valve; 3. Wash-bottle; 4. Connecting piece; 5. Quartz tube; 6. Connecting piece; 7. Absorber; 8. Burner; *a.* Cotton plug; *b.* Copper oxide; *c.* Asbestos plug.

The differences between duplicate analyses are generally less than 1.5 mm.^{3} HCl. If they are greater, which sometimes happens, it is advisable to perform a third and fourth analysis, if the size of the dissolved precipitate allows such a procedure.

As shown in Table II, we have made a series of analyses both on dissolved substances and on organisms. In respect to the former,

TABLE II

Substance in γ	Carbon content in γ		Remarks
	Calculated	Found	
Cane-sugar 106	44.7	43.8	
Cane-sugar 53	22.4	26.6	
Cane-sugar 26.5	12.2	11.3	
Cane-sugar 201	81.2	84.7	
Aspartic acid 42	15.1	12.2	
Aspartic acid 42	15.1	14.4	
Creatinine-alum 72	11.0	12.6	
Urea 188	37.6	40.8	
Yeast 218		20.2	Fresh material, directly determined
Yeast 109		12.2	
Yeast 218		26.9	Fresh material, suspended in sea water, precipitated
Yeast 310		22.6	Fresh material, directly determined
Yeast 310		25.7	Fresh material, precipitated from sea water
Yeast 120		14.0	Fresh material, directly determined
Yeast 120		14.8	Fresh material, precipitated from sea water
Yeast 190		16.0	Fresh material, directly determined
Yeast 190		23.0	Preserved with formaldehyde, precipitated from sea water
Yeast 300		30.0	Fresh material, directly determined
Yeast 300		27.0	Preserved with formaldehyde, precipitated from sea water

Number of organisms in the single determination	Carbon content in γ		Remarks
	Found	Calculated for 100,000 organisms	
253,000 Skeletonema costatum	17.0	6.7	Fresh culture, precipitated
614,000 Skeletonema costatum	29.6	4.7	Fresh culture, precipitated
1,020,000 Skeletonema costatum	46.2	4.5	Preserved with formaldehyde, precipitated
44,500 Thalassiosira Nordenskiøldi	21.8	49.0	Fresh culture, precipitated
70,000 Thalassiosira Nordenskiøldi	39.8	57.0	Preserved with formaldehyde, precipitated
75,100 Thalassiosira Nordenskiøldi	41.0	55.0	Preserved with formaldehyde, precipitated

it must be observed that the small amount of water burned (0.1 ml.) gives a noticeable amount of CO_2, which cannot be neglected. We are unable to explain this blank value. We have found that it is the same whether we use normal distilled or redistilled water. We can only say that it originates in the drying process.

Examples

1. Determination of 26.5γ cane sugar in 0.1 ml. water = 11.3γ C.

Baryta solution directly titrated: 93.6
93.6

93.6 mm.^{3} n/3 HCl

Combustion of 0.1 ml. of the water used: 90.6
91.0

90.8 mm.^{3} n/3 HCl

Combustion of 0.1 ml. of the cane sugar solution: 84.0
85.4

84.7 mm.^{3} n/3 HCl

Titrations of the water: 90.8
Titrations of the sugar solution: −84.7

6.1 mm.^{3} n/3 HCl, corresponding to 12.2γ C

Calculated: 11.3γ C; found: 12.2γ C.

2. Determination of the carbon content of 360,000 *Thalassiosira Nordenskiœldi* (fresh culture).

Weight of the dissolved precipitate containing the organisms: 0.479 gram (=0.48 ml.).
Baryta solution directly titrated: 92.3
92.5

92.4 mm.^{3} n/3 HCl

Combustion of 0.1 ml. of the control precipitate: 89.3
88.4

88.8 mm.^{3} n/3 HCl

Combustion of 0.1 ml. of the organism precipitate: 68.0
68.6

68.3 mm.^{3} n/3 HCl

Titrations of the control precipitate: 88.8
Titrations of the organism precipitate: −68.3

20.5 mm.^{3} n/3 HCl corresponding to 41γ C

These 41γ C belong to the organisms in 0.1 ml. of the precipitate; therefore in the organisms of the whole precipitate we have: 197γ C.

The results of our analyses, as given in Table II, show that the mean accuracy resulting from duplicate analyses is in the case of dissolved substances about ± 2γ C, in the case of organisms about ± 3γ C. The percentage accuracy is therefore in high degree dependent upon how much substance is available for the single determination. It is not advisable to perform analyses with less than 10γ C in the single determination. On the other hand, carbon amounts of about 100γ C can be determined without difficulty.

The method is easy to carry out. It will probably give reliable results, for example, in researches on the metabolism of protozoa, the assimilation of lower algæ, and generally in cases where only small amounts of material are available, but where relatively large differences between the determinations can be expected. Concerning the practical use of the method in researches on the carbon content of small amounts of plankton, all will depend upon how far it will be possible to avoid contamination of the water sample with carbon-containing material (for example: the oil used to lubricate the water bottle).

Acknowledgments

The writer desires to express his sincere thanks to Professor A. Krogh, on whose suggestion this work has been undertaken and who followed it always with helpful criticism. He thanks further Dr. Braarud and Dr. Steemann Nielsen for providing him respectively with diatom cultures and preserved plankton material.

Summary

The method of Krogh and Keys for nitrogen determination is adapted for determinations on plankton. The range of nitrogen to be determined is 0.5–5γ, the accuracy about 10 per cent.

A method for the determination of small amounts of carbon for the same purpose is described. Amounts of 10 to 100γ C can be determined with an accuracy of about ± 3γ C.

BIBLIOGRAPHY

Krogh, A., 1930. Eine Mikromethode für die organische Verbrennungsanalyse, besonders von gelösten Substanzen. *Biochem. Zeitschr.*, **221**: 247.

Krogh, A., 1934. A method for the determination of ammonia in water and air. *Biol. Bull.*, **67**: 126.

Krogh, A., and A. Keys, 1934. Methods for the determination of dissolved organic carbon and nitrogen in sea water. *Biol. Bull.*, **67**: 132.

Krogh, A., and P. B. Rehberg, 1929. CO_2-Bestimmung in der atmosphärischen Luft durch Mikrotitration. *Biochem. Zeitschr.*, **205**: 265.

Krogh, A., and P. B. Rehberg, 1930. CO_2-Bestimmung in Flüssigkeiten und Geweben durch Mikrotitration. *Biochem. Zeitschr.*, **225**: 177.

Steemann-Nielsen, E., and Th. v. Brand, 1934. Quantitative Zentrifugenmethoden zur Planktonbestimmung. *Rapports Cons. perman. internat. explor. de la mer.*, **89**: 99.

Teorell, T., 1932. Eine neue Methode zur titrimetrischen Bestimmung kleinster Mengen Ammoniak. *Biochem. Zeitschr.*, **248**: 246.

THE EFFECTS OF SALINITY CHANGES ON BODY WEIGHT AND SURVIVAL OF NEREIS VIRENS

LEONARD P. SAYLES

(*From the Department of Biology, the College of the City of New York, and the Marine Biological Laboratory, Woods Hole*)

It has been demonstrated by Pearse (1928), Annandale (1922), Vaughn (1919), Ferronierre (1901), and Florentin (1899), among others, that many marine forms will survive for various lengths of time in dilutions of sea water. This survival is made possible by the ability of the organism to adjust itself to its external medium. Many marine animals are ordinarily in osmotic equilibrium with their environment. Accordingly, if a marine organism is to survive in a new environment of altered salinity, it must attain a new equilibrium. Bethe (1930), working with the sea-slug *Aplysia*, has shown that osmotic movement of water is not the only factor in this equilibrium, but that, in addition, movement of salts takes place. Margaria (1931), working with certain crabs and with the dogfish *Scyllium*, noted that these animals soon had blood of the same osmotic pressure as that of diluted sea water in which they were placed. Adolph (1925) has pointed out that fresh-water forms, in their ordinary medium, are not in osmotic equilibrium with their surroundings. Furthermore, there is in fresh-water invertebrates a restricted and selective interchange of inorganic solutes. Marine invertebrates, on the other hand, have less resistance to the penetration of water than have fresh-water forms and therefore adjust themselves more rapidly (Adolph, E. F. and P. E., 1925).

Materials and Methods

As a measure of the adaptability of a marine organism to altered salinity, it is of interest to determine the influence which various dilutions of sea water have on the weight of the body. For this purpose the marine annelid *Nereis virens* was used. Work along this line on two other species of the same genus has been reported by Beadle (1931).

The work here described was done at Woods Hole during parts of several summers between 1929 and 1934, a portion of it by Dr. Nelson F. Waters in the summer of 1930 as a problem in connection with the Invertebrate Zoölogy Course at the Marine Biological Laboratory.

I am indebted to him for the use of the information which he obtained at that time.

The experimental animals, obtained in as fresh a condition as possible, were kept in the laboratory for several days to allow them to clear their digestive tracts. For the experiments, worms were put into finger bowls, one in each with 250 cc. of solution. All dilutions were made with tap water. The water was changed once or twice a day. In any experiment where an animal was changed to new solutions a few hours apart, each of the other worms was changed into a fresh solution of the same concentration as that in which it was found. All weighings were made to the nearest milligram. Previous to weighing, each worm was freed of most of the adherent water by rolling it hurriedly on filter paper. Any changes in the composition of the media were made immediately after the weighings. Worms were not fed during the experiments.

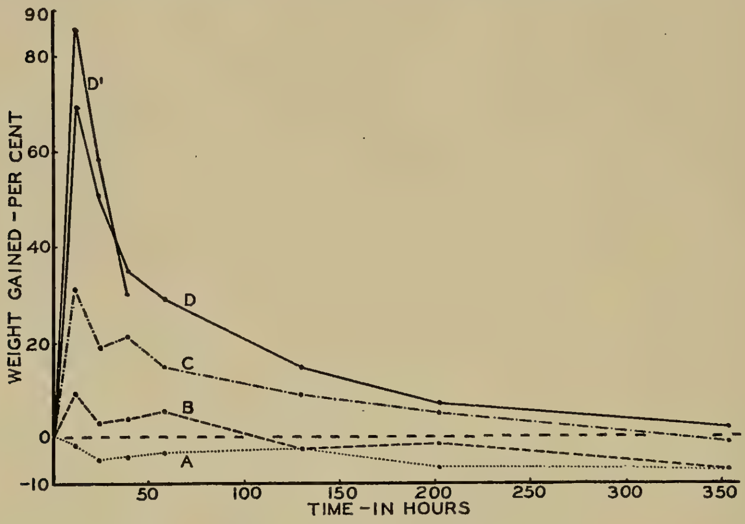


FIG. 1. Changes in body weight of *Nereis* following transfer directly to various dilutions. *A*, in ordinary sea water; *B*, in 4/5 sea water; *C*, in 3/5 sea water; *D* and *D′* in 2/5 sea water.

EXPERIMENTAL RESULTS

Experiment 1. Changes in Body Weight of Worms Following Transfer Directly to Various Dilutions

The results of the longest experiment of this type, continued for 354 hours, are shown in Fig. 1. Five worms were weighed and placed in solutions as follows: *A* into sea water as a control; *B* into 4/5 sea water; *C* into 3/5 sea water; *D* and *D′* into 2/5 sea water. *D′* died after 38 hours. Each experimental animal increased in weight, the one in the greatest dilution showing the greatest increase. Also, after 11 hours, each returned toward its original weight, rapidly at first but more slowly as time went on. In *Nereis virens*, therefore, there was an increase in weight, the greater the greater dilution. Each worm, however, showed a tendency to accommodate itself to its new environment and to return to a weight which approximated that which it had at the start.

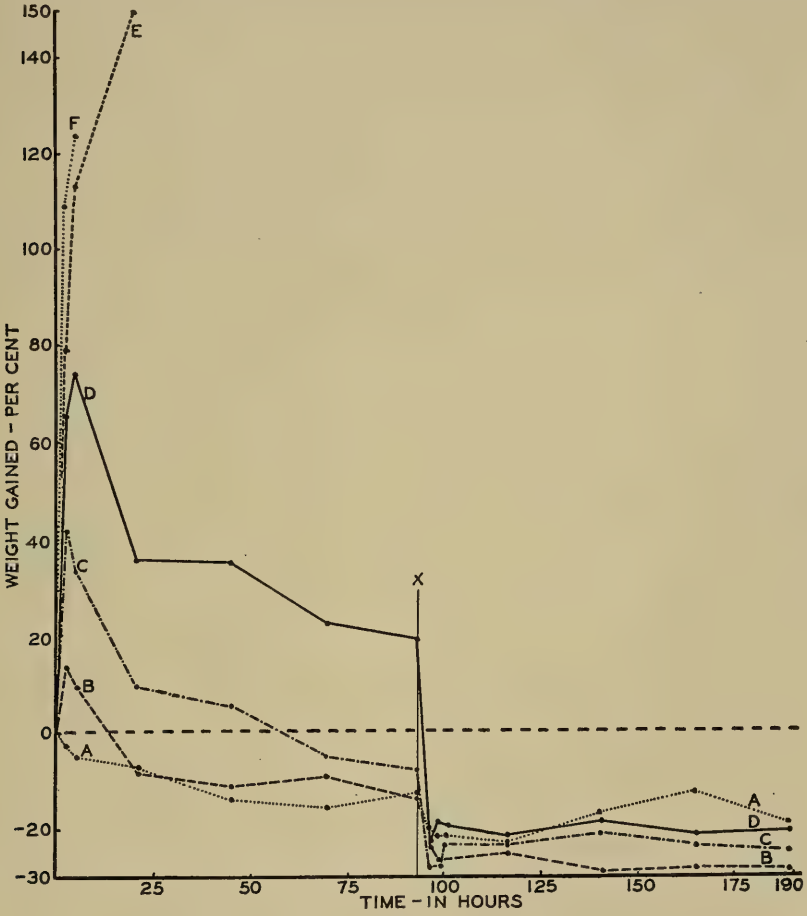


FIG. 2. Changes in body weight following transfer directly to various dilutions and upon return to ordinary sea water from these dilutions. *A*, in ordinary sea water; *B*, in 4/5 sea water; *C*, in 3/5 sea water; *D*, in 2/5 sea water; *E*, in 1/5 sea water; *F*, in ordinary tap water. At *X*, after 93 hours in dilutions, the return to sea water was made.

Experiment 2. Effect of Return to Sea Water after a Period in Dilutions

In an early experiment in this series 6 worms were at first placed in solutions as follows: *A* into sea water; *B* into 4/5 sea water; *C* into 3/5 sea water; *D* into 2/5 sea water; *E* into 1/5 sea water; and *F* into ordinary tap water. Figure 2 gives the data for this experiment.

In the case of the control there was a gradual loss up to 93 hours. A sharp drop then occurred during the next 3 hours. Not infrequently there was some loss of weight when two successive weighings were made within a few hours of one another. During the first half of this experiment there was a close parallel with the results of Experiment 1. Here, however, specimens were also put into 1/5 sea water and straight tap water. Both of these last mentioned worms died during the first day. The weight increase of *E* (in 1/5 sea water) was intermediate between *F* and *D*. *E* also survived several hours longer than *F*.

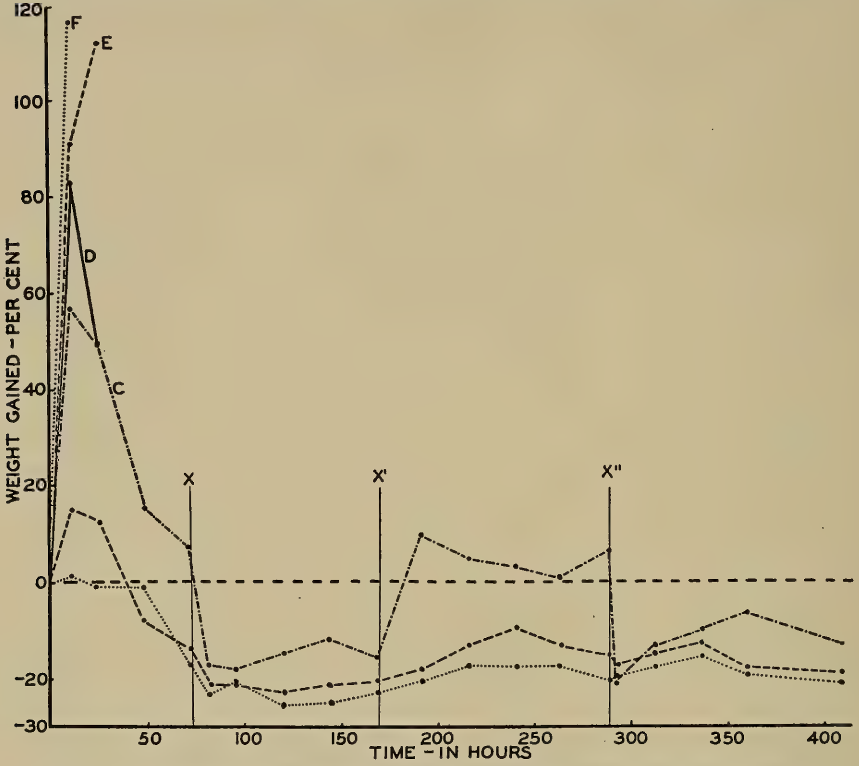


FIG. 3. Effects of return to dilutions after period in sea water and of return to sea water again. *A–F*, as in Fig. 2. Worms were placed in dilutions at beginning of experiment, returned to ordinary sea water at *X*, put into the dilutions again at *X′*, and finally returned to sea water at *X″*.

Another new point brought out by this experiment is that, although in Experiment 1 the apparent maximum increase was at 11 hours, most of the worms had probably begun to lose weight at that time. In the present experiment the worms were weighed at 2½ hours and again at 5 hours. Here the apparent maximum was reached at the first weighing in the cases of *B* and *C* and at 5 hours by *D*. A comparison of all the graphs shows that not only was there a more rapid rise but there was a longer period of rise the greater the dilution.

At the end of 93 hours all were returned to sea water. All the experimental animals showed a drop in weight, a very marked one in the case of *D*. The noteworthy point seems to be that *D* dropped very soon to almost the same level as the others. Although there were some individual variations from this point to the end of the experiment, about 4 days later, the weights of all remained fairly close during this time.

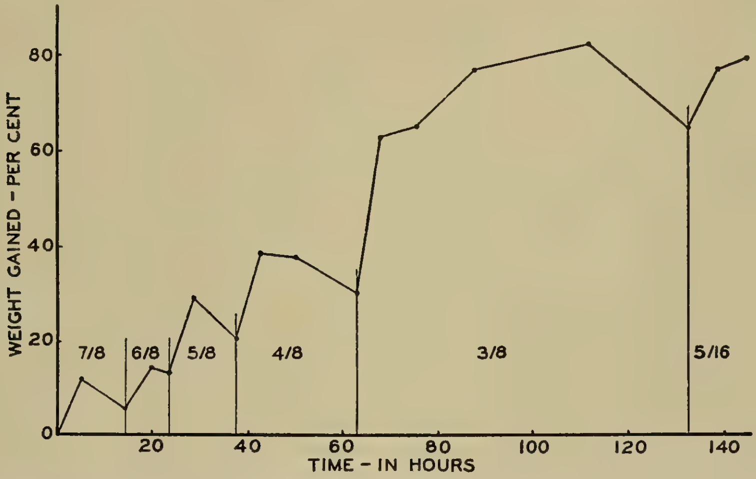


FIG. 4. Effect of gradual dilution of the external medium. Period in each dilution is indicated by the fractions, e.g. 7/8, indicating time in 7/8 sea water.

Experiment 3. Effect of Return to Dilutions after a Period in Sea Water

In this case worms were placed in various dilutions as above. The results are shown in Fig. 3. The responses of these worms to the transfers to dilutions and back to sea water were similar to those in the other two experiments described above.

Ninety-six hours after the return to salt water *B* was transferred back to 4/5 sea water and *C* back to 3/5 sea water. Both increased in weight. *B*, however, just about paralleled the control except for one weighing. *C*, on the other hand, increased rather rapidly to about 25 per cent (using as a basis its original weight) after 24 hours.

After the first transfer to dilute sea water the same worm had increased about 50 per cent after 24 hours.

Five days after the return to the dilutions the worms were again placed in ordinary sea water. The weight of *C* dropped suddenly even below that of the control. Both experimental worms then increased in weight a little and at the end of the experiment they were quite close to the control worm.

Experiment 4. Effect of Gradual Dilution of External Medium

Figure 4 gives an example of a worm which was weighed at least once or twice between successive changes. Each change except the last was, of course, a relatively greater drop in salt content than was the one preceding it. As one might expect from this fact, the increase in weight was greater and the duration of the rise longer each time than it was the time before. Another experiment of this type, in which practically no weighings were made between changes, is shown in Fig. 5. Even here the changes were probably made too often. Unfortunately it became impossible to follow the weights of these worms beyond the fifth day in 1/8 sea water. At that time one was killed. The other four were moved from Woods Hole to New York City. Of these, one lived 10 days in the 1/8 sea water and the other three survived 14 days in that dilution. The fact that the thirteenth and fourteenth days were very warm and that no facilities were available for keeping the water at an even relatively cool temperature

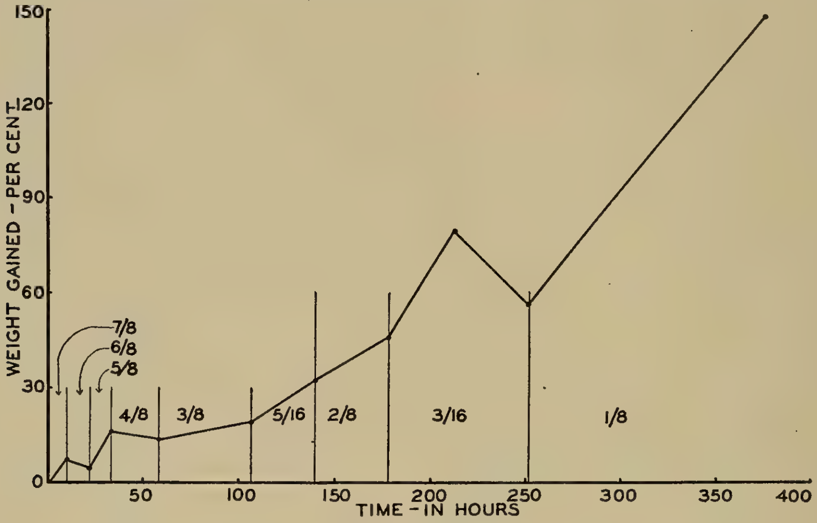


FIG. 5. Effect of gradual dilution of the external medium. Average of 5 specimens. Period in each dilution indicated by the fractions as in Fig. 4.

probably explains the deaths of the last three. In contrast with this survival in 1/8 sea water, *Nereis virens* transferred directly to 1/5 or lower concentrations died within a few hours. Pearse (1928) reported that this species lived 3 hours in 1/8 sea water.

Experiment 5. Effect of Transfer to Fresh Water from Various Dilutions of Sea Water

To determine whether worms which had lived for a time in dilute sea water were better adapted to withstand fresh water than those which

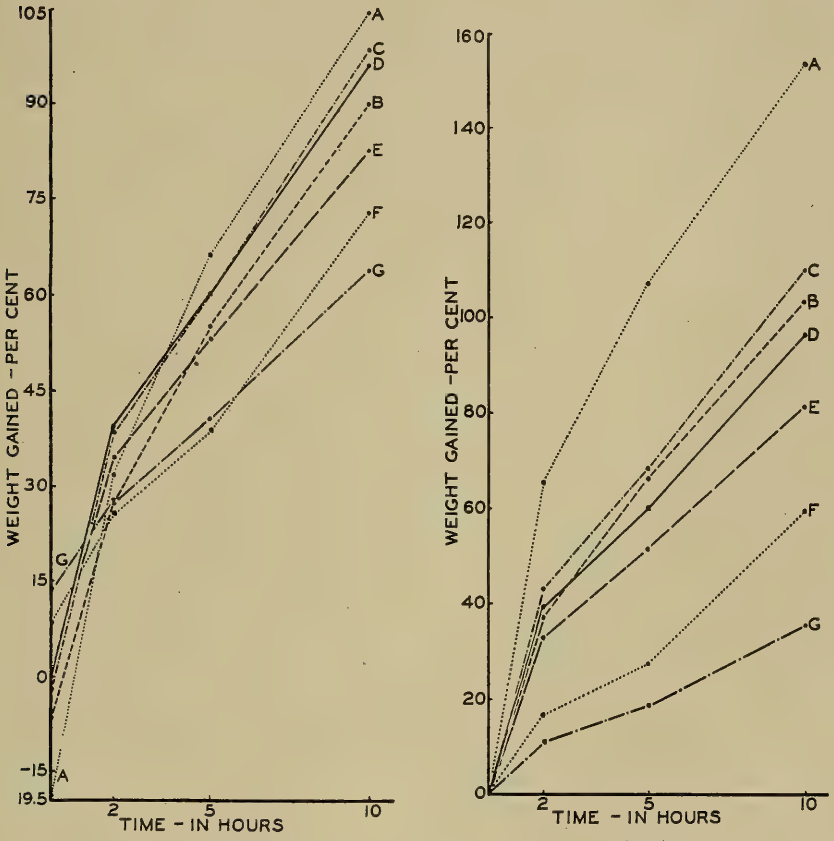


Fig. 6 Fig. 7

FIGS. 6 and 7. Effect of transfer to fresh water from various dilutions of sea water. *A*, from ordinary sea water; *B*, from 7/8 sea water; *C*, from 6/8 sea water; *D*, from 5/8 sea water; *E*, from 4/8 sea water; *F*, from 3/8 sea water; *G*, from 2/8 sea water. In Fig. 6 the weight changes are calculated on the basis of original weights in sea water at beginning of experiment. In Fig. 7 the weight changes are on the basis of weights at the time of transfer of all to the fresh water, 374 hours after the beginning of the experiment.

had been kept in straight sea water, a group of eight worms was treated as follows. After weighing, the control worm (*A*) was kept in sea water and the other seven were transferred to 7/8 sea water. The procedure of gradually changing worms into lower dilutions, as in Experiment 4, was followed here except that at each step one worm was not carried into the next dilution. As a result, at the end of 250 hours, there was the following set-up for the worms in terms of ratio of sea water to fresh water: *A* in 8:0; *B* in 7:1; *C* in 6:2; *D* in 5:3; *E* in 4:4; *F* in 3:5; *G* in 2:6; and *H* in 1:7. Five days later (374 hours after the beginning of the experiment) these worms weighed as follows in terms of percentage of original weight: *A*, 81; *B*, 93; *C*, 98; *D*, 100; *E*, 101; *F*, 109; *G*, 114; and *H*, 266. All were then put into fresh water.

The changes in weight in the fresh water are shown in Fig. 6. *H*, which was very much swollen, burst after the first weighing. In the case of the other worms, there is a practically complete reversal of the order after 10 hours. To show the actual relative increases after transfer from the various dilutions to the fresh water, these same data were recalculated using as a basis the actual weights at the time of this last change. These data are presented in Fig. 7. It may be noted that, during this 10-hour period, there was an increase of but 36 per cent in the weight of *G* and one of 153 per cent in that of *A*.

Further evidence along this same line was given by the behavior of these worms at various times after the transfer to fresh water. In one experiment a worm from 1/8 sea water was still exhibiting slight parapodial movements after 13 hours in fresh water. In worms transferred from ordinary salt water such movements ceased within 1–3 hours. In others, from 1/2 sea water, parapodial movements were not observed after 8–9 hours. It seems, therefore, that worms which have been some time in dilute sea water are able to withstand exposure to fresh water better than those which have been kept in ordinary sea water. These differences might possibly have been due in part to the fact that in the more dilute solutions the body fluids of the animals had lower osmotic pressures due to the loss of salts. Also this difference might have been due to the fact that the mechanism which rids the body of excess water was more actively at work in the low concentrations than in the higher, especially ordinary sea water. In the course of these experiments no attempt was made to discover what this mechanism might be. Although various opinions concerning the nature of this mechanism have been expressed by Schlieper (1930), Adolph (1927), Dakin and Edmonds (1931), Margaria (1931), and others, the problem still remains an open one.

Discussion

A longer continued rise and a greater increase in weight the greater the dilution have been observed by Bethe (1930), working with the sea-slug *Aplysia*. He found increases comparable to those described for *Nereis virens*. The time element, however, was quite different. *Aplysia*, in 50 per cent sea water, returned nearly to its original weight (to about 1.15 ×) in 6–10 hours. *N. virens*, on the other hand, usually required about 60 hours to return to a similar level in 60 per cent and over 100 hours in 40 per cent. *Aplysia*, placed in 75 per cent sea water, returned to its original weight in 4–5 hours and dropped to 0.85 × in less than 8 hours. *N. virens* required, in most cases, more than 30 hours to return to its original weight in 4/5 sea water. Unfortunately Bethe does not report any controls. In experiments begun 2 or 3 days after the worms had been brought into the laboratory, controls of *N. virens* dropped in some cases below 0.8 ×. Also it is known (Adolph, E. F. and P. E., 1925) that temperature changes have a marked effect on changes in body weights of invertebrates. Consequently, in the absence of reported controls, it is not possible to make any satisfactory comparisons of the time factor between Bethe's results and those presented here for *N. virens*.

Beadle (1931) reports on two other species of *Nereis* (*N. diversicolor* and *N. cultrifera*). In 1/4 sea water *N. diversicolor* increased to 1.7 × in 18–20 hours, remained at that level for about 50 hours and then gradually dropped to 1.4× at 120 hours, when the experiment was ended. Speaking of the behavior in 1/6 sea water, Beadle says (p. 216), "Unfortunately, of a number of *N. diversicolor*, all except one shed eggs soon after transference to the dilute water." The weight of the one exception rose to about 2.5 × at 20 hours and then slowly dropped to about 2 × at the end of the experiment. Many specimens of *N. virens* broke after they had increased 2 × to 3 ×. As in the case of the two individuals of *N. diversicolor* indicated in Fig. 2 of Beadle's paper, the specimens of *N. virens* paralleled one another for a time. Then some would begin to decrease in weight while others were gradually increasing. In each case where the weight continued to increase the integument eventually became stretched very thin and the worm became turgid. In many of these instances a rupture of the integument soon occurred with the loss of a large amount of body fluid. Although some of these individuals lived for a long time, it seems better to interpret this effect as due to the skin becoming stretched to a breaking point by the swelling of the organism rather than to attribute it to the ordinary shedding of genital products.

A comparison of these three species of *Nereis* shows that, in the first place, *N. virens* could not withstand as great dilutions as could the other species. Also, in dilutions of approximately minimum survival concentration, the maximum body weight of *N. diversicolor* (about 1.7 ×) was maintained for about 50 hours and that of *N. cultrifera* (about 2.3 ×) for at least 70 hours. *N. virens*, in a corresponding solution, was well on the way to its original weight within 24 hours after the maximum was reached. At 50 hours after the maximum it was down to about 1.3 × and at 70 hours down to a little over 1.2 ×.

Adolph, E. F., and P. E. (1925, p. 108) found that "a brief lowering of the temperature 5° C. led in one hour to a loss of weight of 4 per cent" in the case of *Lumbricus*. In harmony with these data are those reported in Experiment 1 of this paper. A worm of 3/5 sea water increased in weight nearly 4 per cent during a period of 15 hours, when it might have been expected to lose a little over 1 per cent. Thus there was a difference of about 5 per cent between actual and expected weights. During the time of the unexpected increase the laboratory temperature rose from a little below 22° C. to 25° C. Except for this especially warm day the temperature remained at 21°–22° C. during this experiment. At this same time other worms in this experiment also showed an increase, though very slight.

N. virens, when returned to sea water from dilutions, lost weight rapidly. After this initial loss a slight increase in weight occurred. This is in agreement with Bethe's statements (1929 and 1930) that *Aplysia*, returned to sea water from dilutions, exhibited an initial loss of water followed by the intake of salt from the external medium. Adolph (1925, p. 329), on the other hand, reports that in 1/2 sea water *Phascolosoma* "lost slowly a considerable amount of the dissolved substance of the body fluids, so that when finally returned to normal sea water very little water passed from the body compared to that originally gained." It seems to us that loss of salts from the body should help to decrease the osmotic pressure of the body fluids and that, as a result, the ordinary salt water would be relatively higher in comparison. Therefore, one might expect an even greater loss of water from the worm than would result if no such salt-loss occurred. It is clear, however, that although an animal in 2/5 sea water might lose a considerable amount of salts—as found by Bethe (1929) for *Carcinus* and *Aplysia*—its weight did not drop distinctly below the control on return to sea water.

When specimens of *N. virens* were again placed in their respective dilutions they once more increased in weight. These changes were more gradual and smaller than those following the first subjection to dilutions. Perhaps these less marked responses to diluted sea water were due to the loss of salts during the "recovery period" of the first exposure to dilute sea water. In Experiment 2 the worms were returned to ordinary sea water a second time. This time, especially, they showed changes almost the exact opposite of those occurring on transfer to dilutions. There was a sharp loss followed by an increase in weight. Dakin and Edmonds (1931, p. 180) have expressed the opinion that on transfer to dilutions "muscular contraction resulting from *any* unfavorable conditions may cause expulsion of water and consequent loss in volume and weight." Such an explanation could hardly apply to the increase in weight following the initial loss after the return to ordinary sea water. The similarity between this latter response and that shown on the transfer of worms to the dilutions is sufficient, it seems, to raise some doubt as to whether or not the expulsion of water soon after immersion in dilute sea water is due, even for the most part, to muscular contraction.

Adolph (1925, p. 332) states that "most marine animals are able to live after abrupt change to almost pure fresh water, providing that the remaining salts are present in physiological proportions." Although he says that some lived in 2 per cent or even in 1 per cent sea water, he cites no references and names no specific organisms. Certainly such is far from the case with *Nereis virens*, for which we have found the minimum survival concentration to be near 2/5 sea water.

SUMMARY

1. *Nereis virens* increased in weight on transfer to sea water diluted with fresh water. This increase was greater the greater the dilution.

2. The minimum survival concentration was between 30 and 40 per cent sea water. In concentrations of greater salt content than this, *N. virens* decreased in weight after the initial increase. This decrease was rapid for about a day and then gradual.

3. A few scattered observations of body weight changes which seemed to be associated with temperature changes have been made. These are in agreement with the report of Adolph, E. F. and P. E. (1925).

4. On return to sea water from a dilution, *N. virens* lost weight rapidly. This loss was greater the lower the concentration in which the animal had been kept. After this initial loss a slight increase in weight occurred.

5. When specimens of *N. virens* were placed for a second time in their respective dilutions they increased in weight again. This time, however, the changes were more gradual and smaller than those after the first subjection to dilutions.

6. Although *N. virens* survived but a few hours after direct transfer to 1/8 sea water, it lived 14 days in that dilution when transferred slowly through intermediate dilutions.

7. Worms which had been several days in dilute sea water lived in fresh water longer than did worms which had been transferred directly from ordinary sea water. Also the more dilute the solution in which the animal had been kept the less the rise in weight on transfer to fresh water.

LITERATURE CITED

Adolph, E. F., 1925. Some physiological distinctions between fresh-water and marine organisms. *Biol. Bull.*, **48**: 327.

Adolph, E. F., 1927. The regulation of volume and concentration in the body fluids of earthworms. *Jour. Exper. Zoöl.*, **47**: 31.

Adolph, E. F., and P. E. Adolph, 1925. The regulation of body volume in fresh-water organisms. *Jour. Exper. Zoöl.*, **43**: 105.

Annandale, N., 1922. The marine element in the fauna of the Ganges. *Bijdr. Dierk. Amsterdam* (*Feest-num., M. Weber*), p. 143.

Beadle, L. C., 1931. The effect of salinity changes on the water content and respiration of marine invertebrates. *Jour. Exper. Biol.*, **8**: 211.

Bethe, A., 1929. Ionendurchlässigkeit der Körperoberfläche von wirbellosen Tieren des Meeres als Ursache der Giftigkeit von Seewasser abnormer Zusammensetzung. *Pfluger's Arch.*, **221**: 344.

Bethe, A., 1930. The permeability of the surface of marine animals. *Jour. Gen. Physiol.*, **13**: 437.

Dakin, W. J., and E. Edmonds, 1931. The regulation of the salt contents of the blood of aquatic animals, and the problem of the permeability of the bounding membranes of aquatic invertebrates. *Austral. Jour. Exper. Biol. and Med. Sci.*, **8**: 169.

Ferronnierre, G., 1901. Études biologiques sur les Zones supra-littorales de la Loire-Inferieure. *Bull. Soc. Sci. Nat. Ouest Nantes*, **11**: 1.

Florentin, R., 1899. Faune des mares salées de Lorraine. *Ann. Sci. Nat. Zool.*, (8) **10**: 209.

Margaria, R., 1931. The osmotic changes in some marine animals. *Proc. Roy. Soc. London, B*, **107**: 606.

Pearse, A. S., 1928. On the ability of certain marine invertebrates to live in diluted sea water. *Biol. Bull.*, **54**: 405.

Schlieper, C., 1930. Die Osmoregulation wasserlebender Tiere. *Biol. Rev. and Biol. Proc. Camb. Philos. Soc.*, **5**: 309. Excellent Reference List.

Vaughn, T. W., 1919. Corals and the formation of coral reefs. *Smithsonian Reports*, p. 189.

CHLORIDE AND TOTAL OSMOTIC PRESSURE IN THE BLOOD OF MARINE TELEOSTS

ALLAN L. GRAFFLIN[1]

Department of Anatomy, Harvard Medical School

(From the Mt. Desert Island Biological Laboratory, Salisbury Cove, Maine; the Stazione Zoologica, Naples, Italy; and the Laboratory of the Marine Biological Association, Plymouth, England)[2]

Profiting by our previous experience which has demonstrated rather rapid changes in the blood and urine of marine teleosts under experimental conditions (Grafflin, 1931; Pitts, 1934), the present study has been carried out in an attempt to establish, in so far as possible, the range of plasma chloride and of total osmotic pressure in various species under their normal conditions of existence in the sea. A review of the literature necessitated the assumption that, particularly with respect to the osmotic pressure determinations, the figures reported must in many cases, from a consideration of the methods by which the specimens were procured, represent values significantly higher than those obtaining normally. It became of great interest to determine the degree of constancy of the plasma chloride and the blood delta in a given species and in species of widely separated phylogenetic groups, when specimens were obtained under fairly ideal conditions. Throughout this study only those values obtained upon blood drawn immediately from fishes caught with hook and line have been accepted as representing the normal values in a given species.

METHODS

Blood was drawn by hypodermic syringe by three methods, to be referred to simply as heart, sinus, and tail. *Heart:* the heart was exposed, and blood was removed by puncture of the ventricle or of the bulb of the aorta. *Sinus:* the gill covering was held away from the body, and the large venous sinus behind the gill arches was rapidly exposed by incision of the overlying tissues. It is to be noted that specimens of cod, pollack and haddock caught in deep water frequently showed a collection of gas in the sinus. When this was at all marked the fish was discarded. *Tail:* the needle was carried through the fleshy part of the tail from the ventral side and exactly in the mid-line posterior to the urinary papilla (in *Conger* considerably posterior to the papilla, to avoid the caudal kidney) until the firm resistance of the vertebral column was encountered. The needle was then withdrawn very slightly before suction was exerted upon the syringe. With some experience blood can be obtained almost invariably and quite rapidly by this method, which was originally developed by Dr. Homer W. Smith. Any fish found torn and (or) bleeding at the point of hooking was rejected in this study.

---

[1] Fellow of the John Simon Guggenheim Memorial Foundation, 1934.

[2] I wish to express my indebtedness to Prof. Reinhard Dohrn, Director of the Naples station, Dr. E. J. Allen, Director of the Plymouth laboratory, and their staffs for their whole-hearted coöperation and assistance while I was carrying out the present study. Also, it is a pleasure to acknowledge the cheerful coöperation of Captains Bunce and Honey of the "Rachel," whose knowledge and skill made the work at Plymouth possible.

Chloride was determined by the method of Van Slyke (1923) with the modifications reported by Smith (1930), and is expressed throughout as millimols per liter. After the plasma samples had been digested with nitric acid, the tubes were thoroughly cooled in an ice-water mixture, solid ferric alum was added as an indicator, and titration was carried out at once. In the course of the titration ether was added to float the precipitated AgSCN, and near the end-point the tubes were again chilled with ice. Under such conditions, when the tubes were vigorously shaken throughout the titration, the end-points were very sharp and permanent.[3] All determinations were carried out against a standard of 160 millimols per liter, which was quite close to the chloride concentration in the unknown solutions. A standardization was run with each batch of chlorides, the tubes being handled in exactly the same manner as the plasmas. At each of the three laboratories the entire group of chloride determinations was carried out with the same 3 cc. pipette for the silver nitrate solution and the same 0.5 cc. precision pipette for the samples. Comparison with a 1.0 cc. pipette showed that no error was introduced by the use of the smaller samples. Except in a few instances, where insufficient fluid was available, duplicate analyses were made. As a further check upon the method, it was demonstrated that chloride added to plasmas of known concentration could be recovered quantitatively.

Freezing-points were determined on whole blood in an apparatus kindly supplied by Dr. W. R. G. Atkins of the Plymouth laboratory. The size of the samples was approximately 3.5 cc. Inoculation with delicate crystals of hoar-frost was carried out at an average of 0.2° below the freezing-point, and no correction has been made for undercooling. The thermometer was of the Beckmann type, constructed for the freezing-point range, and was read to 0.001° with a hand lens. Check readings on blood samples usually agreed within 0.002°, at the most within 0.004°. The ice-point was determined at the beginning and end of each set of blood determinations, and remained constant. As a further check upon the apparatus, it was shown that the freezing-point of a known sodium-chloride solution could be determined accurately to the second decimal place. These facts, coupled with the consistent checks obtained upon the same blood samples at different times, lead us to believe that the deltas reported are accurate to the second significant figure.

---

[3] I was led into the present problem by a distrust of my earlier plasma chloride analyses (1931), which were carried out without the improvements in the method suggested by Smith. It was apparent that these analyses had almost certainly suffered from overtitration, due to the use of a considerable volume of indicator solution, as recommended by Van Slyke, and to the marked opacity of the fluid at the end-point; and so had given values which were too low, since the error from overtitration would be significantly greater with plasmas than with standard chloride solutions or blanks.

TABLE I

Plasma chloride of various fishes bled immediately after catching on hook and line.* Data obtained at Salisbury Cove, Me.

Myoxocephalus octodecimspinosus (Sculpin) millimols/liter		*Pseudopleuronectes americanus* (Flounder) millimols/liter	*Gadus callarias* (Cod) millimols/liter	*Myoxocephalus scorpius* (Daddy sculpin) millimols/liter	*Gadus pollachius* (Pollack) millimols/liter	*Melanogrammus æglefinus* (Haddock) millimols/liter
158.0	162.8	149.7	150.9	163.1	150.5	150.5
159.2	162.8	152.4	150.9	164.4		152.1
160.0	162.8	153.2	151.3			
160.4	162.8	154.0	151.3			
160.4	163.2	154.4	151.3			
160.8	163.5	154.4	151.6			
161.2	163.6	155.2	155.2			
161.6	165.2	155.7				
162.4	166.4	158.4				
162.4	168.4					

* Sculpins and flounders were taken in Frenchman's Bay, and were bled from the heart; cod, pollack, and haddock were taken near Egg Rock, off Bar Harbor, and were bled from the sinus. Heparin was used as anticoagulant. I wish to acknowledge the assistance of Mr. David Ennis in these experiments at Salisbury Cove.

RESULTS

In Table I are given the normal values for plasma chloride in various teleosts taken at Salisbury Cove, Maine. Sculpins and flounders were caught in rather shallow water and bled very rapidly, and it is reasonable to assume that the values obtained are essentially identical with those existing in the blood immediately before catching. Granting this assumption, it is clear that the sculpin shows a considerable physiological range of variation in the plasma chloride level; and, although the data are not so extensive, this conclusion applies with equal force to the flounder. In the family including the cod (with the exception of one value—155.2 millimols), haddock and pollack, on the other hand, there is indicated an almost rigid constancy of the chloride level. It is to be noted that such constancy has not been borne out for this family by fairly extensive work on pollack at Plymouth. As a first approximation from the data in Table I, it seems that the better protected, scaled members of the cod family are able to regulate chloride at a significantly lower level (average three species 151.6 millimols) than the non-scaled, skin-covered sculpins (average two species 162.5 millimols), and that the flounder (average 154.2 millimols) is intermediate between them in this respect. Further consideration of the possible correlation between integument and chloride level will be deferred until the discussion.

TABLE II

Plasma chloride of fishes bled immediately on removal from the Aquarium, Stazione Zoologica, Naples *

Scorpæna scrofa† millimols/liter		*Trigla corax*‡ millimols/liter		*Sargus rondeletii*‡ millimols/liter	
147.6	150.8	153.1	158.7	155.1	161.5
149.6	151.2	153.9	163.9	160.7	165.1
150.8	157.9	156.0	179.7		
Serranus gigas†		*Labrus turdus*‡		*Cantharus lineatus*§	
155.5		145.2		151.2	161.9
Serranus cabrilla‡		*Muræna helena*‡		*Pagrus vulgaris*†	
158.6		156.7		163.0	
				Pagellus erythrinus‡	
				167.4	

* Heparin used throughout as anticoagulant. † Bled from exposed heart. ‡ Bled from tail with exception of *Trigla* No. 3 (156.0), which was bled from tail and heart. § Bled from tail and heart.

That it is necessary to obtain blood under ideal conditions to arrive at the normal chloride level is indicated by the following data. Specimens of *M. scorpius* are relatively rare, and *nine* additional specimens, taken in the course of the summer and bled only after a variable delay, sometimes involving extra handling, gave plasma chloride values of 163.2, 166.0, 168.0, 168.6, 168.7, 168.7, 169.8, 174.0 and 177.2 millimols. Three flounders, kept in a live car for some time after catching, gave values of 173.0, 174.0 and 179.0 millimols.

At Naples it was completely impossible to obtain specimens from the sea with hook and line, and Dr. Dohrn was kind enough to allow me to remove from the aquarium many fishes whose long period of residence indicated thorough acclimatization. These fish were netted or hooked from the tanks as they swam about and blood was obtained as soon as possible. The data are summarized in Table II. It is obviously impossible to be sure that the values obtained represent the chloride levels in the blood under normal conditions of existence in the sea, but there are reasons for believing that, on the whole, the figures are probably not far in error. Thus the average for *Scorpœna scrofa* (151.3 millimols) is quite low, and close to the lowest average obtained in the entire study (150.6 millimols). The single specimen of *Pagellus erythrinus* is quite close to the average for specimens of *Pagellus centrodontus* (168.9 millimols) taken under ideal conditions at Plymouth; and the value for *Labrus turdus* is one of the three lowest obtained in the entire study (see Table IV). It must be recalled that the salinity of the Mediterranean is considerably higher than that of the Atlantic Ocean, and that the circulating water of the aquarium tends to be still higher. Comparing the data of Table II with Table I, from the standpoint of chloride level *Scorpœna scrofa* is to be compared with the members of the cod family; *Sargus rondeletii* (which has a very high hematocrit) is to be compared with sculpin (much lower hematrocrit); and *Trigla corax* (if we disregard the value of 179.7 as abnormally high, as has been done in computing the average for Table VI) is intermediate between the flounder and sculpin.[4] To be compared with the low values on aquarium specimens of *S. scrofa* are the following very high values obtained for five living specimens caught fast in a fine net (rete di posta, Stellnetz) which had been in the water eight hours before hauling: 173.8, 180.8, 180.8, 182.1 and 184.3 millimols.

Advantage was taken of an opportunity to obtain specimens of the relatively rare *Orthagoriscus mola* from a large tunny-net off the island of Prochida, near Naples, in an attempt to establish the normal level of plasma chloride in this species. Unfortunately, under the conditions of hauling the net, it was impossible to remove specimens for bleeding singly and while the net volume was still large. For this study the fish were removed rapidly with a hand net or by hand as soon as they were within reach and before the net was too closely hauled, and transferred to reed baskets of good size hung over the side of the ship. The animals were then bled from the exposed heart as quickly as possible (Table III). In computing the average for Table VI, the first five specimens under *A* and all of Group 1 under *B*, Table III, were used, and we believe that the value given (163.5

---

[4] Since Table VI will be referred to several times in this section, it may be stated here that it is a summarizing table in which many of the species studied are gathered together for comparison on the basis of average plasma chloride and average delta.

millimols) is probably not far from the normal chloride level. In the sixteen fish under *A*, Table III, there was considerable overcrowding in the baskets, and that there was a slow, but definite, rise in plasma chloride under these conditions is shown, we believe, by the following averages: Specimens 1 to 5, time after removal from net 5 to 15 minutes, average chloride 163.0; 6 to 10, 15 to 25 minutes, 165.2; 11 to 16, 25 to 35 minutes, 169.7 millimols. On the other hand, the average of Group 2, bled one hour after removal from the net, but not subjected to much overcrowding in the baskets, is not much higher than that of Group 1: 1—164.0; 2—166.0. Two specimens allowed to asphyxiate in the air until dead, and bled 40 minutes after removal from the net, gave plasma chloride values of 167.9 and 168.2 millimols.

Table III

Orthagoriscus mola—Plasma chloride

A. Transferred from close-hauled tunny-net to two reed baskets in water, eight to each basket; bled from exposed heart at regular intervals, in order given; first bled at 5 minutes, last at 35 minutes after removal from net; first basket emptied before starting on second.

1. 160.3
2. 162.4
3. 160.3
4. 167.4
5. 164.8
6. 162.4
7. 167.8
8. 166.7
9. 163.4
10. 165.8
11. 169.3
12. 170.2
13. 167.0
14. 168.8
15. 171.3
16. 171.3

B. Transferred as above, six to each basket; bled from heart in order given; three from each basket for Group 1 (one lost), three for Group 2; Group 1, 4 to 12 minutes after removal from net; Group 2, 61 to 69 minutes.

Group 1
1. 163.9
2. 161.8
3. 163.6
4. 162.6
5. 168.1

Group 2
6. 163.1
7. 168.7
8. 165.1
9. 166.2
10. 163.1
11. 169.5

Table IV contains the data obtained on various teleosts at Plymouth. Certain explanatory details are necessary for the proper interpretation of this table, and are given below.

Explanatory Details for Table IV.—All specimens reported in this table were taken at a considerable distance from shore, in the vicinity of Eddystone Light, with the exception of the group of mackerel upon which chloride alone was determined; this latter group was taken in Plymouth Sound. All hook and line specimens were caught by expert fishermen and hauled into the boat for bleeding as soon as possible. The mackerel, which were of full size, were caught in the usual manner, by dragging lines with the boat in motion. To avoid error, specimens were accepted for bleeding only when they were actually felt to take the hook while the line was being tended. Pollack and conger were taken in from fifteen to twenty-five fathoms of water and were of large size, with an average length of 80 cm. and 125 cm. respectively. Bream and wrasse were large, while the specimen of pout was rather small (all taken in deep water). A considerable number of pollack and conger were taken from the long line (or trawl line), in order to evaluate the degree of abnormality caused by this method of catching. The long line is a stout cord, anchored and buoyed at both ends, and furnished with baited hooks at intervals throughout its length. The line was left overboard for from one to three hours before hauling, and fish were bled as soon as they came on board.

Specimens of pollack, mackerel, bream and pout were bled from the sinus; wrasse and conger (with one exception—Cl 185.6 millimols—heart) were bled from the tail. In one value reported for bream (Cl—170.0 millimols) the blood from two specimens was pooled. Potassium oxalate was used as anticoagulant in the pollack and mackerel upon which plasma chloride alone is reported; heparin was used in practically all other specimens. The exceptional cases are marked with an asterisk and in these no anticoagulant was employed. Since heparin was used in almost all bloods used for osmotic pressure determinations, and since, the fish being taken at sea, there was considerable delay before the freezing-points could be determined, it was necessary to prove that neither of these conditions affected the accuracy of the results. Studies made upon conger and pollack blood demonstrated that the presence of heparin and a delay for periods up to twenty-four hours made absolutely no difference in the results when the bloods were packed with ice as soon as they were drawn. This latter procedure was employed for all samples.

Examination of Table IV shows that in hook and line specimens of pollack, mackerel, and conger there is considerable variation in both plasma chloride and delta, with no correlation between the two. This variation is tentatively interpreted as physiological (see discussion below). It seems entirely justifiable to consider one of the pollack (Cl—173.5 millimols) as highly abnormal, and this fish has not been included in determining the average for Table VI. From the standpoint of plasma chloride, the low average level of pollack agrees with that previously found in the cod family; and mackerel, with its significantly higher level, closely corresponds to the data on sculpin (Table I). In the conger the average chloride is higher than in any other species examined in the entire study, and is approached only by the bream, on which, however, sufficient data are not available. From the standpoint of average delta, pollack is lowest and conger is quite high,

TABLE IV

Plasma chloride and Δ of whole blood. Data obtained at Plymouth.

Gadus pollachius (Pollack)		
1. Hook and line		
Plasma chloride	Plasma chloride	Δ of whole blood
millimols/l.	*millimols/l.*	°*C.*
145.6	—	−0.657
146.7*	—	.673
147.5	146.0	.660
148.3	147.2*	.651
149.0	149.2	.684
149.0	149.9	.657
149.8	150.3	.697
149.8	150.7	.657
151.4	151.1	.690
152.6	152.2	.660
	154.0	.649
	154.1	.683
	158.8	.680
	160.8	.698
	173.5	.778
2. Long line		
155.3	160.7	.739
164.3	162.6	.747
165.4	164.2	.751
167.7	166.2	.800
176.2	168.7	.760
	173.5	.808

Conger vulgaris (Conger eel)			
1. Hook and line		2. Long line	
Plasma chloride	Δ of whole blood	Plasma chloride	Δ of whole blood
millimols/l.		*millimols/l.*	
157.7	−0.728	181.2	−0.778
167.7	.760	182.2	.840
170.7	.762	182.7	.875
171.6	.782	183.3	.813
172.7	.727	185.6	.820
174.2	.788	186.8	.836
175.4	.800	187.6*	.891
175.5*	.765	188.1	.834
176.6*	.749	188.7*	.858
177.3	.794		
179.6	.760		

Scomber scombrus (Mackerel)	
Hook and line	
Plasma chloride	
millimols/l.	
158.4	163.8
159.2	164.2
159.2	164.2
160.0	164.2
161.8	169.2
162.7	170.0
Plasma chloride	Δ of whole blood
157.3	−0.721
157.3	.773
157.7	.754
158.6	.777
159.3	.734
162.4	.775
163.2	.753
165.1	.729
Hook and line	
Pagellus centrodontus (Bream)	
Plasma chloride	Δ of whole blood
165.8	−0.784
170.0	.785
170.9	.815
Labrus bergylta (Ballan wrasse)	
158.8	.682
Gadus luscus (Pout)	
144.1	—

corresponding roughly to their chloride levels, but the delta for mackerel is significantly higher than would be expected from the chloride comparisons alone. In this respect the following percentages, calculated from the averages in Table VI, and expressed in terms of increase above the level found in pollack, are interesting: mackerel, Cl—7.5 per cent, delta—12.1 per cent; conger, Cl—14.6 per cent, delta—14.0 per cent. Whether the relatively high delta of mackerel is associated with its unusually high hematocrit we cannot say. The same consideration applies to the bream.

Specimens of pollack and conger taken on the long line show considerably higher values than those taken on hook and line, and indicate at once the uselessness of material obtained in this way in any attempt to establish normal levels. The following supplementary data serve to reinforce this conclusion. Six mackerel, in excellent condition, were taken from the large weir off Salisbury Cove, Maine, and brought to the laboratory in a large tub, with a constant change of water. They were then bled from the sinus and gave plasma chloride values of 171.1, 171.5, 173.5, 174.7, 176.2 and 182.2 millimols. Also, one specimen of bream taken on the long line showed a chloride of 184.8 millimols.

TABLE V

Comparison of blood from sinus or heart, and tail. Data obtained at Plymouth.

Gadus pollachius	Plasma chloride	Δ of whole blood	*Conger vulgaris*	Plasma chloride	Δ of whole blood
	millimols/l.	*° C.*		*millimols/l.*	*° C.*
Sinus	160.7	−0.739	Heart	185.2	−0.784
Tail	159.3	.774	Tail	187.2	.787
Sinus	154.0*	.649	Heart	186.5	.838
Tail	152.2	.673	Tail	186.8	.838
Sinus	149.9*	.657	Heart	190.0	.840
Tail	146.8	.660	Tail	188.1	.834
Sinus	—	.657	*Gadus morrhua*		
Tail	149.1*	.673	Sinus	150.0	.737
			Tail	150.0	.739

For the sake of maximum speed in obtaining blood, it was necessary to bleed specimens of conger from the tail rather than from the heart. All other adequately studied species were bled from the sinus or heart. Hence, a direct comparison of the conger with the other fishes is justified only if it can be shown that samples of blood drawn from the heart and tail of this species are essentially identical. That this is true for conger is evident from the figures in Table V. Also, a single specimen of *Gadus morrhua* showed identity of the two blood samples. In three out of four specimens of pollack, on the other hand, the delta of tail blood was found to be significantly higher than that of blood from the sinus. From the data at hand, this higher delta is definitely not accompanied by an elevation of the plasma chloride level. Specimens marked with an asterisk in Table V were taken with hook and line, all others on the long line.

In the course of this study, the striking observation has been made upon certain of the Labridæ that the plasma contains a blue or green pigment. We have not been able to pursue this observation further, but in view of the interest of such a finding, the following meagre data are reported: *Labrus turdus* (2 specimens, Naples), plasma green, integument predominantly green; *Crenilabrus pavo* (1 specimen, Naples), plasma blue, integument predominantly blue; *Labrus bergylta* (Plymouth): Specimen No. 1, plasma blue turning to green on standing, integument predominantly orange; Specimen No. 2, plasma green, integument predominantly brown with some blue.

Discussion

An effort has been made in this study to obtain data upon the plasma chloride and total osmotic pressure of the blood of marine teleosts under their normal conditions of existence in the sea. It will now be profitable to examine the results, in an attempt to evaluate the degree to which this aim has been realized. Specimens of sculpin, daddy sculpin, flounder, and mackerel were all caught in shallow water, offered no resistance, and the interval between hooking and bleeding was exceedingly short. The mackerel is at all times a very fast swimmer, and when he is following the bait there is certainly no increase in activity which might be reflected in the blood. Under the circumstances, it is entirely reasonable to conclude that the data on these four species actually represent the *normal* values. Granting this conclusion, it is at once apparent that the plasma chloride level in sculpin, flounder, and mackerel shows an appreciable physiological range of variation, being in no sense rigidly controlled in all individuals of a given species. In mackerel there is also a considerable physiological variation in the delta, and there is no detectable correlation between the delta and the chloride concentration.

Specimens caught in deep water present a particular problem in view of the possibility that, after the fish is hooked, the blood may undergo a significant alteration, as a result of struggling, excitement, impeded respiration or changes in hydrostatic pressure, in the relatively long trip to the surface. A marked release of gas from the blood is frequently observed, and the question arises as to whether one can ever obtain for study a truly normal deep water fish. In an attempt to evaluate the degree of normality of our hook-and-line specimens of cod, pollack, conger, etc., certain facts are pertinent. (1) The specimens of cod, haddock, and pollack reported in Table I, belonging to the same family and all taken in the same fishing period, show, with the exception of one cod (Cl—155.2 millimols), a remarkably constant chloride level (150.5—152.1 millimols). Although such constancy has not been observed elsewhere in the study, it is nevertheless quite improbable that the blood could have been altered significantly in the hooking-bleeding interval and still give such consistent results. (2) Three fishes which had been living for a long period in one of the tanks at Plymouth yielded the following data: pollack, Cl 151.8 millimols, $\Delta - 0.643$; conger No. 1, Cl 171.8 millimols, $\Delta - 0.760$; conger No. 2, $\Delta - 0.743$. These animals had not been molested and could hardly be considered abnormal, and the figures are entirely consistent with the data on hook-and-line specimens given in Table IV. (3) The variation in the plasma chloride level and delta in the hook-and-line specimens of pollack and conger is of the same order of magnitude as that observed in sculpin, flounder, and mackerel, in which any significant abnormality seems to be out of the question. It is obviously impossible to be sure that our data on chloride and delta in relatively deep water forms represent the normal, pre-hooking levels in the blood. On the other hand, in an attempt to obtain normal levels, hook-and-line specimens seemed to offer by far the best type of material. All things considered, it is my belief that the figures reported do not diverge significantly from the normal, and the subsequent discussion of these forms (cod, pollack, conger, etc.) is based upon the tentative acceptance of this conclusion.

In both conger and pollack, which have been rather extensively studied, it is apparent that both the plasma chloride and the delta show a quite appreciable physiological variation, and that in neither species is there any detectable correlation between the two. The strikingly close agreement of the chloride in six out of seven specimens of cod (Table I) is noteworthy, and has not been found in any other species in this study. It will be interesting to see whether a more extended investigation of this species will bear out the almost rigid constancy of chloride which is suggested by the data. One fact which is perhaps significant is that the specimens of cod were relatively small, while the pollack taken at Plymouth were almost uniformly very large. The few data on bream, wrasse, pout (Table IV) and haddock (Table I) are reported without further comment.

In view of the variation in the plasma chloride from species to species, it is of interest to group together, on the basis of *average* chloride level, a number of the fishes which have been examined. This has been done in Table VI, in which only those species of which fairly adequate hook-and-line material has been available are printed in black-faced type. In the case of the few species from the Naples aquarium and of *Orthagoriscus mola*, which have been included, the possibility for deviation from the normal is presumably greater than for hook-and-line material. The ultimate decision with regard to these species must await further work.

A question which naturally arises, in connection with the data in Table VI, is whether there is any possible correlation between the type of integument and the plasma chloride level. For the present it is impossible to make any definite statement on this matter, but there is an indication that perhaps some fairly satisfactory correlation may eventually be forthcoming. Thus the first eight fishes (through *Scomber scombrus*) are all scaled, while four of the remaining five species are skin-fishes, with no scales whatsoever. In this latter group the exception is *Pagellus centrodontus*, which is entirely covered with fairly heavy scales.[5] In view of the data on chloride and delta reported here, it seems that a reconsideration of the effective integumentary insulation of the marine teleosts might be highly profitable.

TABLE VI

Average Plasma Chloride and Average Δ of Whole Blood

Species	Plasma chloride *millimols/liter*	Δ of whole blood ° *C.*
Gadus pollachius	150.6	−0.671
Melanogrammus æglefinus	151.3	
Scorpœna scrofa	151.3	
Gadus callarias	151.8	
Pseudopleuronectes americanus	154.2	
Trigla corax	157.1	
Sargus rondeletii	160.6	
Scomber scombrus	161.9	−0.752
Myoxocephalus octodecimspinosus	162.4	
Orthagoriscus mola	163.5	
Myoxocephalus scorpius	163.8	
Pagellus centrodontus	168.9	−0.795
Conger vulgaris	172.6	−0.765

A critical review of the extensive literature upon the measurement of osmotic pressure and the mechanisms by which it is regulated in the marine teleosts is definitely outside of the scope of this paper, and will not be attempted. One of the aims of this investigation has been to establish beyond question the fact that if one wishes to study osmotic pressure and chloride regulation in this group of fishes, one must obtain his material under ideal conditions. The consistently higher values, as compared with hook-and-line material, obtained on fishes caught with the long line and the net, or bled only after delay and handling, have adequately demonstrated this fact. These higher values likewise emphasize the possibility of serious error in studies of the relative tonicity of blood and urine. It is commonly stated that the urine of marine teleosts is isotonic or hypotonic to the blood (for discussion see Smith, 1932). Suppose that a fish has been caught on the long line, by net or by steam trawl, or that it has been subjected to rough handling after catching. By the time it is bled, the delta of the blood has become elevated considerably above the normal level. The bladder urine, on the other hand, would for the most part have been elaborated by the kidneys from the blood at its normal, significantly lower delta. As a result, the degree of hypotonicity indicated by the figures obtained would be markedly erroneous. The criticism that this source of error has been neglected can unquestionably be applied to many of the data upon this problem in the literature.

---

[5] It is definitely stated in the Cambridge Natural History that in the marine *Conger* of the English coasts "scales are really absent."

Finally, it is interesting to speculate as to the mechanism by which the delta and plasma chloride become elevated in fishes taken on the long line. In view of the considerable degrees of blood concentration which have been demonstrated with muscular exercise in the higher animals, it seems entirely reasonable to believe that the activity involved in struggling on the hook is predominantly responsible for the observed rises in the fishes. This we accept as the most likely explanation. An alternative possibility is that with struggling there is an increased swallowing of sea water, and that increased absorption of the contained salts in the intestine is reflected in the higher chloride and delta of the blood. However, the relative rapidity of the upward shift in the blood seems to be against the acceptance of this latter mechanism.

SUMMARY

An effort has been made in this study to obtain data upon the plasma chloride and total osmotic pressure (delta) of the blood of marine teleosts under their normal conditions of existence in the sea. For this purpose hook-and-line specimens, bled immediately after catching, have been used as the best available material. Studies upon pollack, cod, sculpin, flounder, mackerel, and conger indicate an appreciable physiological range of variation in both chloride and delta, with no detectable correlation between the two in any given species.

The average plasma chloride for many species studied has been found to vary from 150.6 millimols (pollack) to 172.6 millimols (conger) per liter. Supplementary chloride data upon specimens removed from the Naples aquarium and upon *Orthagoriscus mola* (from a large tunny-net) are reported.

It has further been demonstrated that both plasma chloride and delta are as a rule considerably elevated in fishes caught on a long line or by net, and in fishes bled only after a delay and rough handling. It is emphasized that this represents a serious source of error in studies upon normal osmotic pressure relationships and upon the relative tonicity of the body fluids in marine teleosts.

Certain of the Labridæ have been found to contain in their plasma a blue or green pigment.

LITERATURE CITED

GRAFFLIN, A. L., 1931. Urine flow and diuresis in marine teleosts. *Am. Jour. Physiol.*, **97**: 602.

PITTS, R. F., 1934. Urinary composition in marine fish. *Jour. Cell. and Comp. Physiol.*, **4**: 389.

SMITH, H. W., 1930. The absorption and excretion of water and salts by marine teleosts. *Am. Jour. Physiol.*, **93**: 480.

SMITH, H. W., 1932. Water regulation and its evolution in the fishes. *Quart. Rev. Biol.*, **7**: 1.

VAN SLYKE, D. D., 1923. The determination of chlorides in blood and tissues. *Jour. Biol. Chem.*, **58**: 523.

SALT REQUIREMENTS AND ORIENTATION OF LIGIA IN BERMUDA. III[1]

T. CUNLIFFE BARNES

(*From the Osborn Zoölogical Laboratory, Yale University*)

In the first two papers of this series (Barnes, 1932, 1934) the longevity of the littoral isopod *Ligia baudiniana* in various salt solutions was described and it was shown that on land *Ligia* orients toward the sea by positive geotropism. The present report deals with further experiments on salt effects and with the ability of the animal in the sea to orient towards the shore.

HABITS

It was previously reported that no submerged specimens had been found, but during the past summer special attention was paid to very young specimens recently released from the brood pouch (Fig. 1) and occasionally these were found submerged under stones at high tide. The young are probably released in the sea or near the water line and are too small to migrate landwards with the older specimens as the tide advances although they readily leave the water. When kept in a bowl of sea water with a projecting stone, the very young individuals usually remain in the air at the water line, but unlike the fully grown specimens they occasionally remain under water for some hours.

One female released the brood while in air over filter paper moistened with sea water, but all other "births" occurred in submerged specimens. With each vigorous swimming movement, four of five young are expelled.

The tendency of the older animals to avoid entering the water is seen in their leaping from one stone to another. The isopod sways back and forth for a few seconds and then springs across the intervening water—the longest leap observed was 12½ cm.

Further observations were made on the functions of the isopods and their long spines. Besides serving as swimming fins, and to convey water to the gills by capillarity, they are equally effective in draining excess water from the body after the animal has been submerged. After submergence the animal lowers and spreads the uropods in a slow rhythm. The water is pressed out of the gill region by bringing the gill plates close to the body and out again several times. The uropods are separated and brought together in a slow rhythm and lowered to the substratum. At times the last pair of legs are used to remove water film between uropods. If water is dropped on the head or back of an isopod it collects on the underside, runs back to the gills, and is drained onto the ground by the lowered uropods and spines.

---

[1] Contribution from the Bermuda Biological Station for Research.

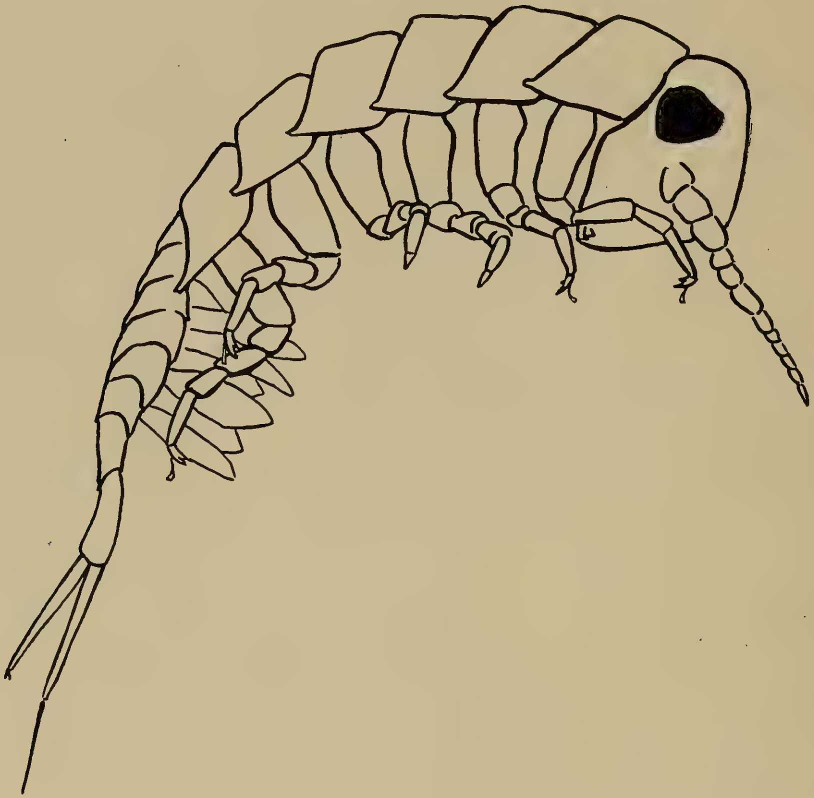


FIG. 1. Young specimen of *Ligia baudiniana* immediately after release from the brood pouch. Note the similarity to the adult animal (*cf.* Barnes, 1932, Fig. 1), except for one missing thoracic segment.

It was previously shown (Barnes, 1934, Fig. 1) that *Ligia* can elevate its gills out of a shallow solution for aerial respiration when the gill beat is inhibited by potassium. The same reaction was observed in natural sea water in an aquarium. A specimen feeding at the water's edge continued "browsing" under water for a short distance but elevated its abdomen out of the water so that the gills remained in air.

Orientation in the Sea

It is very striking to watch the direct line to the shore followed by specimens placed in the sea within ten feet of the land. This is not an orientation to the general direction of the mainland for in tidal pools or cement enclosures the animal swims to the nearest massive solid structure projecting above water.

A plate of glass was placed between the shore and the point of release of the animals in the sea but it had no effect on the direct shoreward migration. The isopods swam frantically against the glass.

If an isopod is placed in a round flask of sea water immersed in the sea it still attempts to swim in the direction of the shore. Regardless of the position of the flask the animal points to the land and its maintenance of direction resembles that of a compass needle. If the flask is placed in a large box open on one side, the swimming movements are directed away from the direction of the light.

From the above experiments it appeared that the animals were reaching away from the light in their landward orientation. It was thought that a large mirror, 3 × 4 ft., immersed in the sea between the land and the point where the isopods were placed, would prevent the direct landward orientation. It was found that under these conditions the animals swam diagonally to shore, arriving at two points on each side of the mirror. An occasional specimen swam out to sea. The mirror experiment was repeated under several conditions with the sun in various directions. It appears that the animal when immersed near the shore is negatively phototropic to the diffuse light in the seaward direction.

Reaction to Drops of Salt Solutions

As pointed out above, when a drop of sea water is placed on the head of *Ligia* it flows under the body to the gills and is drained off by lowering the uropods and spines. The uropods and, to a less extent, the body are moved rapidly from side to side to facilitate the movement of the water. If a drop of 5/8 M NaCl is placed on the head a similar reaction occurs but more slowly and without the side to side movement. It required four drops of $CaCl_2$ to elicit the response. It is difficult to obtain the reflex with 5/8 M KCl. The isopod moves back suddenly and may eliminate the drop by lowering the head. A drop of $MgSO_4$ also causes *Ligia* to move back quickly but the uropodal reflex may be elicited after three drops.

REACTION TO FILTER PAPER SATURATED WITH SALT SOLUTIONS

The specimens used in the tests described in this paper were caught under stones at low tide in the intertidal zone on Long Bird Island. Large filter papers (diameter 25 cm.) were cut in two, each half saturated with a different solution, drained, and placed in a flat glass vessel in a dark room having a light exactly over the center of the dish. Five adult isopods were washed in distilled water (as in all the experiments), allowed to drain themselves on filter paper and placed in the dish. The distribution of the five specimens on the two halves of filter paper was observed every 10 or 30 minutes for 1 to 3-hour intervals. The vessel was rotated 45°, 90°, or 180° between many of the trials to eliminate an unsuspected influence on orientation. In Table I the ratio of the number of individuals on each half of the filter paper is given. In some cases the same isopods were tested several times, which is indicated in the last column in Table I giving the number of specimens tested. Under the conditions of the experiment the animals avoided filter paper wet with distilled water for dry filter paper but collected on filter paper wet with distilled water when the other half was wet with sea water. As will be seen in the table, the animals were found mostly on the sea water or distilled water half of the filter paper when the other half contained a single salt, KCl, NaCl, $CaCl_2$, NH_4Cl and LiCl, all M 5/8. It is of interest to note that there was a tendency to collect on KCl paper when the other half contained NaCl in spite of the fact that KCl is the most toxic and NaCl the least toxic of the common salts in sea water. It was

TABLE I

Reaction to Filter Paper Saturated with Salt Solutions
(each trial involved five animals)

Treatment of each half of filter paper	Ratio of number of isopods on each half	Number of trials	Number of specimens tested
Distilled water vs. dry paper	0 : 10	2	5
Distilled water vs. sea water	9 : 1	4	10
Sea water vs. 4/5 M NaCl	14 : 1	3	5
Sea water vs. 5/8 M KCl	2 : 1	12	15
Distilled water vs. 5/8 M KCl	3 : 1	18	20
5/8 M NaCl vs. 5/8 M KCl	1 : 2	13	20
Sea water vs. 5/8 M $CaCl_2$	2 : 1	2	5
Sea water vs. 5/8 M $MgSO_4$	2 : 1	4	5
Distilled water vs. 5/8 M NH_4Cl	2 : 1	3	5
Distilled water vs. 5/8 M LiCl	5 : 1	4	10

observed that the animals on the KCl showed signs of partial paralysis of the legs and the first pair where held up off the paper. On the other hand, they moved more rapidly on the NaCl paper.

Longevity of Ligia on Filter Paper Saturated with Salt Solutions

Since *Ligia* is very seldom immersed in sea water under natural conditions, the longevity of the animals was determined in finger bowls containing filter paper saturated in various salt solutions. As will be seen in Table II, the animals live for long periods in air on filter paper soaked in distilled water, in sea water, and in artificial sea water. The average longevity of 240 hours in air over filter paper moistened with sea water approaches the average length of life of 375 hours in air over sand moistened with sea water.

Table II

Longevity of Ligia on Filter Paper Saturated with Salt Solutions

Solutions on filter paper	Average longevity	Maximum longevity	Number of specimens
	hours	*hours*	
Distilled water	151.2	216	9
Sea water	240.0	360	15
Artificial sea water	218.4	312	20
100 parts 5/8 M NaCl, 2.5 parts 5/8 $CaCl_2$	168.0	240	6
100 parts 5/8 M NaCl, 2.5 parts 5/8 M $MgSO_4$	121.4	192	16
100 parts 5/8 M NaCl, 2.5 parts 5/8 M KCl	112.8	168	14
5/8 M NaCl	72.0	120	8
5/8 M $CaCl_2$	41.1	60	8
5/8 M NH_4Cl	36.0	72	10
5/8 M $MgCl_2$	30.0	50	8
5/8 M KCl	30.0	58	14
5/8 M LiCl	3.5	6.5	10

The order of toxicity of 5/8 M single salts on filter paper is Li > K, Mg > NH_4 > Ca > Na, similar to the toxicity series for immersion in these solutions. On filter paper saturated with a mixture of 2.5 cc. of a 5/8 M solution of a given salt and 100 cc. of 5/8 M NaCl the toxicity series is K > Mg > Ca.

The filter paper experiments afforded conditions not unlike those provided by confining an isopod in a dry bowl containing a watch glass of salt solution. The position of NH_4Cl and $MgCl_2$ were determined in the toxicity series under these conditions. With access to NH_4Cl, 10 specimens lived an average of 3 hours with a maximum of 7 hours and 16 specimens with access to $MgCl_2$ lived an average of 18 hours and a maximum of 38 hours, thus making the toxicity series $NH_4 > K > Mg > Ca > Na$ for the watch glass series.

LONGEVITY OF LIGIA IMMERSED IN SOLUTIONS OF SINGLE SALTS

The position of NH_4 and Li in the toxicity series was determined in view of their physiological similarity to K and Na respectively. In 5/8 M NH_4Cl ten specimens tested separately in finger bowls containing 100 cc. of solution lived an average of 27 minutes with a maximum of 35 minutes. Like KCl, NH_4Cl paralyzed the beating of the gills. The LiCl had to be used sparingly in smaller vessels containing only 30 cc. of solution. For comparison with the other salts it was necessary to repeat all the salt effect tests in 30 cc. samples. It will be seen from Table III that the toxicity series for these salts in 5/8 M concentration is $NH_4 > Li > K > Mg > Ca > Na$. The shorter life of isopods in 30 cc. samples compared to 100 cc. samples is probably a result of the depletion of oxygen. This factor is probably not important in the case of 100 cc. of solution in a shallow finger bowl, as is indicated by the longevity of specimens in 100 cc. samples which were bubbled with air. The length of life in the aerated solutions (Table IV) is the same as previously determined in unaerated solutions of 100 cc. volume.

TABLE III

Longevity of Ligia in 30-cc. Samples of Single Salt Solutions

Medium	Average longevity	Maximum longevity	Number of specimens
Distilled water	3 hours	4 hours	5
Sea water	8.2 "	30 "	10
5/8 M NaCl	4.5 "	10 "	5
5/8 M $CaCl_2$	2 "	3 "	5
5/8 M $MgSO_4$	1.4 "	2 "	7
5/8 M KCl	1 "	2 "	5
5/8 M LiCl	42 minutes	60 minutes	10
5/8 M NH_4Cl	22 "	30 "	5

The position of NH_4 Cl and LiCl was also determined in the toxicity series for dilute solutions of single salts containing 2.5 cc. of 5/8 M concentration in 100 cc. of distilled water which is near the concentration of K and Ca in sea water. In 2.5 cc. of 5/8 M NH_4Cl in 100 cc. of H_2O 10 specimens lived an average of 1.3 hours with a maximum of 2 hours; in the same concentration of LiCl the average longevity of 10 specimens was 2.4 hours with a maximum of 2.5 hours.

TABLE IV

Longevity of Ligia in 100 cc. of Solutions Bubbled Continuously with Air

Medium	Average longevity	Maximum longevity	Number of specimens
	hours	*hours*	
Distilled water	4.2	5	8
25% sea water	6.9	14	24
5/8 M NaCl	8.0	10	8
5/8 M $CaCl_2$	6.6	10	5
5/8 M $MgSO_4$	4.6	5	4
5/8 M KCl	1.3	1.5	2

The toxicity series of salts in this weak concentration may be written $NH_4Cl > LiCl > NaCl > KCl > CaCl_2$.

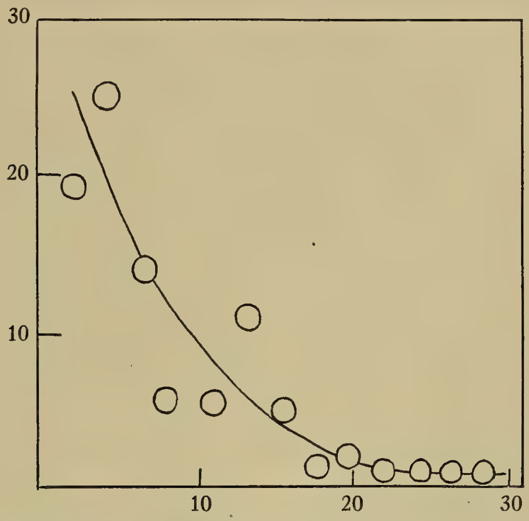


FIG. 2. Length of life of *Ligia* in 100 cc. 5/8 M NaCl+ 11.6 cc. 5/8 M $MgSO_4$ with increasing amounts of KCl. Ordinates, life in hours. Abscissæ, cc. of 5/8 M KCl added to 100 cc. 5/8 M NaCl. Each point is the average of 2–4 tests.

EXPERIMENTS WITH SALT MIXTURES

Since the degree of toxicity of the chief cations in sea water is not the same, it is of interest to determine if the normal concentration of a given salt in sea water is near its threshold concentration for shortening the life of immersed specimens. However, individuals vary to a great extent in their survival times in sea water and we have not yet completed a sufficient number of experiments. The rapidly increasing toxicity of artificial sea water without Ca in which the KCl content is progressively increased may be seen in Fig. 2. When the normal K content is increased fourfold, a marked curtailment of the length of life is obtained and when the K content is increased tenfold the average survival time is only one hour. A somewhat similar curve was obtained for artificial sea water containing Ca but in this case some of the animals live as long as 90 days in the solutions containing less than the normal amount of K, which renders the variation in longevity so great that more tests will have to be run. However, in the artificial sea water containing Ca, toxic effects appear if the K content is four times that of normal sea water and paralysis of the gill beats occurs when the K content is increased to 17 times the normal amount (i.e., 37.4 cc. 5/8 M KCl, 100 cc. 5/8 M NaCl and 2.5 cc. 5/8 M $CaCl_2$). In binary solutions of NaCl and KCl the gill paralysis appears when the K content is only ten times the normal value.

TABLE V

Longevity of Young Ligia in Salt Solutions*

Medium	Average longevity	Maximum longevity	Number of specimens
Distilled water	2 hours	3 hours	15
25% sea water	12.8 "	24 "	16
5/8 M NaCl	4.5 "	8 "	16
5/8 M $CaCl_2$	1 "	1.1 "	55
5/8 M $MgSO_4$	.75 "	1 "	7
5/8 M KCl	25 minutes	25 minutes	18

* These animals were tested immediately after their release from the brood pouch.

LONGEVITY OF YOUNG SPECIMENS IN SALT SOLUTIONS

Young specimens were tested in salt solutions immediately after their release from the brood pouch. The young are about 2.5 mm. in length and have only six thoracic segments (Fig. 1). According to Calman (1909, p. 213), the young of all Isopoda have the last pair of thoracic limbs undeveloped. As will be seen in Table V, the toxicity series for the chief cations in sea water is the same as that found with adult specimens but the survival times are shorter. The tests were made under the microscope in watch glasses, each containing three individuals.

DISCUSSION

The finding of specimens recently released from the brood pouch, totally submerged in the sea, supports the view that *Ligia* begins its life history under water, as is indicated by the large number of "births" that occurred in sea water or other salt solutions during the experiments. However, the toxicity series for the chief cations in sea water is the same for young and mature specimens.

Although it is impossible to state what factors are responsible for the evolution of this marine form into a littoral species, it appears from the orientation studies that the animals are confined to the shore zone by positive geotropism from the land and by a negative reaction to the greater diffuse light from the open ocean when immersed near the shore. It is perhaps significant that the isopods react more favorably to filter paper saturated with distilled water than to filter paper saturated with sea water. However, when kept for long periods on filter paper saturated with various solutions, the animals live longest on paper wet with sea water.

Numanoi (1934) finds that *Ligia exotica* cannot regulate the evaporation of water from the body and therefore can live only in the vicinity of water.

It is significant that the toxicity series $K > Mg > Ca > Na$ for 5/8 M solutions previously determined for immersed specimens and for individuals having access to watch glasses of solutions was obtained for animals kept on filter paper saturated with salt solution. These experiments demonstrate the specific toxicity of each salt apart from permeability or osmotic effects, and afford more natural conditions than the immersion tests. Heilbrunn (1928) emphasizes the necessity of allowing for valency differences when using $NaCl$ and $CaCl_2$ solutions, but the specific chemical effects of 5/8 M solutions of the chief cations in sea water on *Ligia* are much more marked than the effects of slight osmotic changes and, moreover, there were obviously no osmotic effects in the filter paper experiments.

The paralyzing action of NH_4Cl on the gill beat affords a new instance of the physiological similarity of NH_4 and K and supports the hypothesis that the biological effects of ions are in part determined by their mobility. The ions K and NH_4 have high mobilities (65.3 and 64.2) because of their small shell of water molecules (*cf.* Maass and Steacie, 1931, p. 220). Wells (1928) has pointed out the similar effects of NH_4 and K on the muscles of invertebrates. Potassium has a specific toxic action, apart from the paralysis of the gills, for in artificial samples of sea water in which it is increased, the shortening of the average longevity of the animals occurs at potassium concentrations only four times that of natural sea water but the gills are not inhibited until the potassium content is increased about twenty times.

It is interesting to note that the potassium content of sea water is not far from the threshold for definite toxic effects.

SUMMARY

1. In the sea *Ligia* orients toward the shore. The greater diffuse light from the open ocean appears to be the most important factor.

2. Young specimens recently released from the brood pouch were found in the sea.

3. When kept on filter paper moistened with salt solutions *Ligia* shows the same toxicity series as when immersed in solutions or kept in air with access to the solutions.

4. NH_4Cl, like KCl, exerts a specific paralyzing effect on the gill movements.

5. Increasing the potassium content of artificial sea water fourfold or more has a marked effect in shortening the life of immersed animals.

It is a pleasure to express my gratitude to Dr. J. F. G. Wheeler, who placed the facilities of the Bermuda Station at my disposal.

LITERATURE CITED

Barnes, T. C., 1932. Salt requirements and space orientation of the littoral isopod Ligia, in Bermuda. *Biol. Bull.*, **63**: 496.

Barnes, T. C., 1934. Further observations on the salt requirements of Ligia in Bermuda. *Biol. Bull.*, **66**: 124.

Calman, W. T., 1909. In "A Treatise on Zoölogy" (R. Lankester, editor). Part VII. London.

Heilbrunn, L. V., 1928. The Colloid Chemistry of Protoplasm. Berlin.

Maass, O., and E. W. R. Steacie, 1931. Principles of Physical Chemistry. New York.

Numanoi, H., 1934. Relation between atmospheric humidity and evaporation of water in Ligia exotica. *Jour. Fac. Sci. Tokyo Imp. Univ.*, **3**: 343.

Wells, G. P., 1928. The action of potassium on muscle-preparations from invertebrates. *Brit. Jour. Exper. Biol.*, **5**: 258.

STUDIES ON THE SECONDARY SEXUAL CHARACTERS OF CRAYFISHES: X. THE ANNULUS VENTRALIS IN TRUE INTERSEXES OF CAMBARUS

C. L. TURNER

(*From the Zoölogical Laboratory, Northwestern University*)

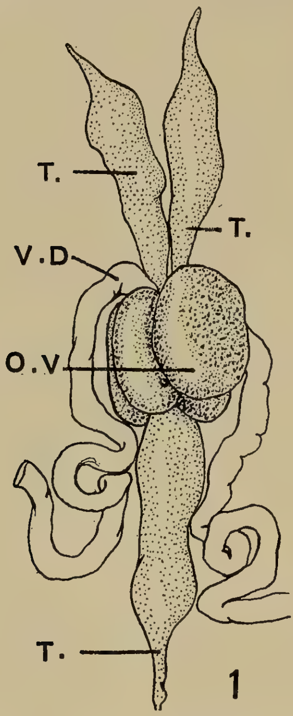
The external secondary sexual characters in the genus *Cambarus* are so constant in structure and position that they are used extensively for sub-genus and species diagnosis. Some variation has been found in structure, number, and position of these characters, however, in many instances characters of one sex being found on members of the opposite sex. Such variations occur principally in copulatory hooks, oviducal pores, first and second abdominal appendages and, to a lesser extent, in the male genital pores. Variations in the annulus ventralis are extremely rare, records of only four cases being found in the literature. Two additional specimens were recently collected by E. P. Creaser on June 29, 1932. Both specimens were found in one locality, the Huron River, five miles southwest of Dexter, Michigan. These latter two are the only ones seen by the writer in examining more than 75,000 females of the genus *Cambarus*.

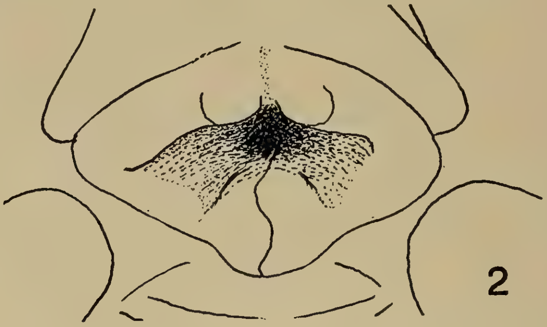
DESCRIPTION OF SPECIMENS

Specimen No. 1 is 42 mm. in length. The following external features are those of a male: 1. First abdominal appendages are male-like but are somewhat shorter and more slender in the basal half than in the normal male. 2. Second abdominal appendages are specifically modified like those of a male but shorter. 3. Normal genital pores of male are at the base of the fifth leg.

The specimen has the following external secondary sex characters of a female: 1. No copulatory hooks are present on the third legs. 2. An oviducal pore occurs on the left third leg, but it is not perforated and is not surrounded by hairs. 3. An annulus ventralis is present, but the lateral walls have not formed so that the anterior and posterior walls are separable and movable. Three pencils of stiff hairs are present upon the low, partially formed posterior wall. In the normal annulus the posterior wall is smooth but bristles are present in this region in the normal male.

The internal reproductive organs are those of a normal male with the addition of some scattered patches of ovarian tissue (Fig. 1). The gonad and vas deferens were examined by sectioning and they proved to be normal in every respect. A four-lobed ovarian mass was located dorsal to the isthmus of the testis and two other isolated patches appeared, one adhering to the ventral surface of the heart to the left and anterior to the main mass of the ovary, and a second to the right





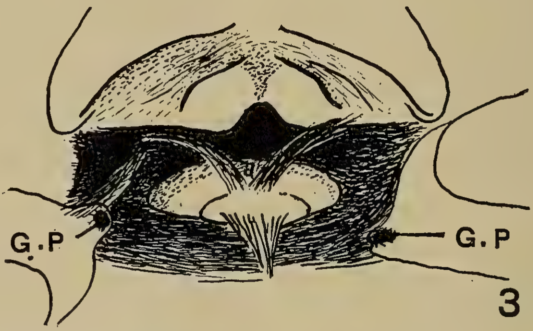


FIG. 1. Reproductive organs of Specimen No. 1.

Ov. Ovary
T. Testis
V. D. Vas deferens

FIG. 2. Annulus ventralis of normal female of *Cambarus propinquus*.
FIG. 3. Partially formed annulus ventralis of Specimens No. 1 and No. 2.

and posterior to the main mass. All the masses were identified as ovarian tissue by sectioning.

Specimen No. 2 is 46 mm. in length. It is identical with Specimen 1 except in the following regards: 1. There is no oviducal pore on either third leg. 2. The second abdominal appendages are somewhat better developed. 3. The internal ovarian patches are smaller and more scattered.

Discussion

Since variations in the annulus ventralis are so rare, it is thought best to bring together all the described cases including the two new ones presented in this paper. Table I presents the information in a form that will readily permit comparison of the different features in the different specimens.

Six specimens is a very small number upon which to base generalizations, but to one who is acquainted with the vagaries of unusual secondary sex characters, some facts appear which are unique and are believed to be significant.

The case of the specimen of *C. spinosus* is not held to be particularly unusual and should probably be classified with those instances in which a normally functioning female has developed first and second abdominal appendages of the male. At least 25 female specimens have been described in which one or both of the first two abdominal appendages are male-like. The slightly flattened annulus ventralis is characteristic of all females after they have been freed from their young and have moulted the winter shell. The annulus of this specimen is probably a little lower than usual for that reason.

In the other 5 specimens it should be noted that the presence of the partially developed annulus is accompanied by a teratological hermaphroditism in at least two of the cases. In the specimen described by Hay the preservation was so poor that nothing could be determined and the two described by Ortmann were not dissected. The two specimens are undoubtedly true intersexes. The terms "hermaphrodite" and "intersex" have been used repeatedly to describe specimens of *Cambarus* in which secondary sex characters of one sex are found upon the opposite sex, but in only one other specimen (*C. affinis* described by Hay) has it been demonstrated that both ovarian and testicular tissue were present. The impression is created, therefore, that the partially developed annulus ventralis is associated with a true intersex condition.

A second significant fact is that all five of the specimens have the partial development of some secondary sex characters of both sexes but not one has a complete complement of either male or female secondary sex characters. With the exception of these cases, the many specimens with aberrant secondary sex characters that have been examined by the writer have always had a full complement of secondary sex characters of one sex. The peculiarities of the secondary sex characters have consisted of the addition to this complete complement of one sex of one or more characters of the opposite sex. Copulatory hooks

TABLE I

Species	Copulatory hooks	Annulus ventralis	Oviducal pores	Male genital pores	Abdominal appendages	Spermary	Ovary
C. spinosus.....	Normal for male	A little lower than usual	Normal for female	Imperforated papillæ	First and second normal for male	None	None
C. bartoni......	None	Juvenile type	None	Present but indistinct	First normal for male; second as in normal female	Not dissected	Not dissected
C. obscurus.....	None	Indistinct with outline and median depression visible	None	Normal for male	First male-like but short; second normal for male	Not dissected	Not dissected
C. propinquus...	Normal for male on left side; none on right	Lower than normal; hairs on posterior wall as in male	Normal for female on right side; none on left	Normal on left side; absent on right side	First and second normal for male on left side; first shorter than normal male and second as in female on right side	Poor condition; not positively identified	Poor condition; probably normal ovary
C. propinquus...	None	Anterior and posterior walls partly formed; lateral walls lacking; hairs on posterior wall	Present on left third leg but not perforated; absent on right side	Normal for male	First and second like male but shorter and slender	Normal	Small isolated patches of ovarian tissue
C. propinquus...	None	Anterior and posterior walls partly formed; lateral walls lacking; hairs on posterior wall	None	Normal for male	First and second like second-form male but short and slender	Normal	Small isolated patches of ovarian tissue

are always present in males and oviducal pores always occur in females. In a great many instances copulatory hooks occur upon females and occasionally oviducal pores are found in males, but in no other instances except these five has there been found the partial or complete lack of both oviducal pores and copulatory hooks. A normal annulus ventralis is always present in functional females of *Cambarus* and as far as the writer is aware no case has ever been recorded of the occurrence of an annulus ventralis in a normal functional male. The occurrence of the partially developed annulus ventralis and the lack of copulatory hooks and of oviducal pores in these five cases would therefore support the point of view that neither sex had been fully determined but rather that an intersex condition existed.

It might be postulated upon the evidence offered in these cases that an antagonistic hormonal mechanism exists here, a hormone from the testis inhibiting a full development of female secondary sex characters and a hormone from the ovary preventing the normal growth and differentiation of the male sex character. However, such a mechanism would not be consistent with that governing those cases in which secondary sex characters of one sex are found upon normally functioning animals of the other sex. Oviducal pores are sometimes fully developed in normal males where the postulated antagonistic hormone of the testis should be most effective. Copulatory hooks are frequently found in fully functioning females and, to a lesser extent, male abdominal appendages as well. If the ovary produced an antagonistic hormone, the development of these male structures in the female should have been inhibited. An alternate point of view to that of an hormonal relation existing between gonad and secondary sex characters would be the theory that the secondary sex characters are controlled only by genetical factors. In these five cases a rather close relationship would be indicated between the factors for sex and those governing the secondary sex characters, especially in the case of the annulus ventralis.

LITERATURE CITED

HAY, W. P., 1905. Instances of hermaphroditism in crayfishes. *Smithsonian Misc. Coll.*, **48**: 222.

ORTMANN, A. E., 1906. The crayfishes of the state of Pennsylvania. *Mem. Car. Mus.*, **2**: 343.

TURNER, C. L., 1924, 1925, 1928, 1929. Studies on the secondary sexual characters of crayfishes, I to IX. *Biol. Bull.*, **46**: 263; **48**: 225, 232; **49**: 375, 379; **55**: 193, 197, 255; **56**: 1.

INDIRECT EFFECTS OF RADIATION ON SEA URCHIN EGGS[1]

L. V. HEILBRUNN AND R. A. YOUNG

(*From the University of Pennsylvania and the Marine Biological Laboratory, Woods Hole, Mass.*)

Students of the effects of radiation have now and again sought to explain their findings in terms of humoral physiology. The best known authority in the field is Caspari (*cf.* Caspari, 1926), and he has introduced the term "necrohormones" into the literature. Caspari believes that certain cells are more sensitive to radiation than others, and that these more sensitive cells when radiated produce necrohormones which affect other cells remote from them. Unfortunately, however, Caspari has offered very little in the way of clear-cut direct evidence in favor of necrohormones, and many radiologists are inclined to doubt their existence. Thus, in the treatment of tumors, although some authors (Kok, 1924; Regaud, 1924; Waterman and de Kromme, 1927; de Guide, 1933) believe that radiation may have important indirect effects, others (Wood, 1925; Jolly, 1924; Lacassagne, 1928; etc.) insist that all the therapeutic action of radiation is due to a direct effect on the cells involved. It is obvious that the solution of the problem may have practical clinical significance. As yet, however, radiologists have not brought forward much in the way of convincing experimental evidence. This is in part due to the difficulty of working with the complicated organs or tissues of higher animals, in part also to the difficulty of evaluating quantitatively the effects of radiation on cells.

Sea urchin eggs are very favorable material for the study of rœntgen ray effects. They have been used by Mavor and de Forest (1924), H. and M. Langendorff (1931), and especially by Henshaw (1932). If eggs are irradiated and then inseminated, there is a delay in development. By noting the time of first cleavage, Henshaw was able to study this delay quantitatively.

The original plan of our experiment was to study the effect of roentgen rays on concentrated and dilute suspensions of eggs. Our primary concern was not in the clinical aspects of the problem, but we were interested in discovering the possible presence of so-called necrohormones produced as a result of the radiation. It was thought

---

[1] Aided by a grant from the Committee on Radiation of the National Research Council.

that the irradiation of the more concentrated suspensions might cause a greater delay in cleavage time than similar treatment of dilute suspensions. A few experiments of this sort were tried, but the differences between the two types of suspensions were not very striking. It was then found that if ovarian tissue was irradiated with eggs, a greater effect was produced than in controls in which the eggs were irradiated alone.

The work was done at the Woods Hole Marine Biological Laboratory during the summer of 1934. The Laboratory rœntgen equipment was the same as that used by Henshaw, a Coolidge, tungsten target, air-cooled tube. The rays were not filtered (except by cellophane; see below). The apparatus was in charge of Mr. M. Sander. The tube was maintained at 125 kv. and 5 ma. and the measured intensity was found to be 850 r.p.m., when the eggs were 19 cm. from the target.

The general plan of the experiment was very simple. Two (or in some cases three) vials were placed under the rœntgen tube. These vials were approximately an inch in diameter and they were cut down to a height of approximately an inch and a quarter. One or more ovaries of the sea urchin, *Arbacia punctulata*, were placed in one vial. The eggs oozed out of the ovary to form a concentrated suspension. To this a small amount of sea water was added. A second vial served as a control. It contained either a dilute or concentrated suspension of *Arbacia* eggs, but in this case no ovarial tissue. In order to prevent evaporation, the vials were covered with cellophane. As soon as exposure to the radiation was completed, the eggs from both vials were washed free of blood and they were then inseminated. Between the end of the exposure period and insemination about 3–5 minutes elapsed. The time intervening between insemination and first cleavage, that is to say the cleavage time, was determined by noting the moment when 50 per cent of the eggs showed the beginning of a cleavage plane. This was compared with the cleavage time for control non-irradiated eggs, and the difference was taken as the delay due to irradiation. In determining the cleavage time for non-irradiated eggs, two lots were used. In one of these lots, the eggs had been in a dish with ovarial tissue in the same manner and for the same length of time as those eggs irradiated in the presence of ovary. The second lot consisted of eggs alone. Before insemination the eggs were washed free of blood. Actually there was little difference in cleavage time between eggs which had been in the presence of ovarian tissue and those which had not, but in every case the delay caused by irradiation was calculated by comparison with control eggs treated in identical manner except for the irradiation.

Table I shows the results of the experiment. The first column gives the dose in *r* units, the second column shows the delay in cleavage time of eggs irradiated in the presence of ovary. Columns 3 and 4 show delays obtained with dilute and with concentrated suspensions irradiated in the absence of ovary. In every case, a markedly greater delay is obtained when ovary is present. The differences are certainly significant and are all in the same direction. In view of the fact that the experiments were run at different room temperatures, and that, moreover, various lots of eggs may differ in their behavior, comparisons should only be made across the table, that is to say, for the same lot of eggs in each case.

TABLE I

Dose in *r* units	Delay in minutes		
	Eggs + ovary	Dilute suspension	Concentrated suspension
17,000	35	20	
17,000	55	28	
17,000	39	13	
25,500	81	17	
25,500	46	18	
25,500	32	15	
25,500	42	33	
25,500	40	24	
25,500	45	32	
25,500	35		12
25,500	45		28
25,500	36		29
25,500	28		22
25,500	27		19
25,500	18		10
51,000	110	25	
51,000	70	25	
51,000	64	46	51
51,000	76	26	40
51,000	46	37	

Table I shows clearly that when the eggs are irradiated along with ovarian tissue, more pronounced effects are produced than when no ovary is present. Apparently, therefore, irradiated ovarian tissue produces substances which affect the egg cells. It might be thought that other organs or tissues of the sea urchin would also give off substances when irradiated and that these substances would act in the same manner as those produced from ovaries. Experiments were tried with blood (cœlomic fluid) and with the digestive tract. In these experiments, eggs in contact with blood or digestive tract were irradiated and the effects of the irradiation compared with the effects of similar doses on control eggs in the absence of other tissue. The blood was not found to have any definite action, and the digestive tract either had no significant effect or perhaps an effect in the opposite direction from that exerted by the substances from ovarian tissue. Thus in one experiment eggs irradiated in the presence of stomach and intestine segmented before those irradiated alone. The only conclusion that can be drawn from these very scanty observations is that under the conditions of the experiment neither blood nor alimentary tract produced substances capable of increasing the delay produced by irradiation of the eggs alone.

That other organs of the sea urchin do not share the action of the ovaries in increasing the rœntgen ray effect is indicated (though not proven) by the results obtained from irradiating entire sea urchins. Table II summarizes the data from these experiments and compares them with the figures already given for eggs alone and for eggs plus ovary. In the experiments on whole sea urchins, the animals were irradiated intact, then at the end of the exposure the eggs were cut out and inseminated. Normal control eggs had to be obtained from another female. Owing to variation between eggs from different females, this introduces a source of error. Delay in cleavage time was obtained by comparison of eggs from irradiated animals and normal control eggs.

TABLE II

Delay in Cleavage Time under Different Conditions of Irradiation

	Dose in *r* units	Delay in minutes (average of 12 experiments)
Ovary + eggs	25,500	39.6
Eggs alone	25,500	21.6
Entire sea urchins	27,700	27.4

The experiments with entire sea urchins cannot be certainly interpreted as indicating the lack of effect of organs other than ovaries. It is to be noted that the irradiation of entire sea urchins has less effect in delaying cleavage than the irradiation of eggs in the presence of ovaries. However, when the entire animal is irradiated, the calcareous shell doubtless shields the eggs to some extent. The effect of this shell could be quantitatively estimated by direct experimentation, but this was not attempted.

The experiments on irradiation of eggs in the presence of ovary indicate that ovarian cells when exposed to rœntgen rays produce a substance or substances which act on the eggs. More convincing proof of the presence of such necrohormone-like substances might be obtained from the study of the effect on eggs of sea water which had been in contact with irradiated ovaries. It might indeed be possible to isolate the necrohormone in a relatively pure state. Experiments in this direction are planned for the immediate future.

SUMMARY

When sea urchin eggs are exposed to rœntgen rays in the presence of ovarian tissue, the effect of the radiation is more pronounced than when the eggs alone are irradiated.

LITERATURE CITED

CASPARI, W., 1926. Physiologie der Röntgen- und Radiumstrahlen. *Bethe's Handbuch der norm. und path. Physiol.*, **17**: 343.

DE GUIDE, C., 1933. Contribution a l'étude de l'action indirecte des radiations. *Bruxelles-Médical*, **13**: 1309–1318, 1327–1336, 1407–1416.

HENSHAW, P. S., 1932. Studies of the effect of rœntgen rays on the time of the first cleavage in some marine invertebrate eggs. I. Recovery from rœntgen-ray effects in Arbacia eggs. *Am. Jour. Rœntgenol. and Radium Ther.*, **27**: 890.

JOLLY, J., 1924. Action des rayons X sur les cellules. Modifications de la radiosensibilité par ligature des connexions vasculaires. *Compt. rend. Soc. Biol.*, **91**: 351.

KOK, F., 1924. Biologische Versuche über die Wirkung der Bestrahlung auf das Karzinom. III. *Strahlentherapie*, **17**: 134.

LACASSAGNE, A., 1928. The direct and indirect action of radiation on cancer tissues. *Radiology*, **11**: 393.

LANGENDORFF, H., AND M. LANGENDORFF, 1931. Strahlenbiologische Untersuchungen an befruchteten Seeigeleiern. *Strahlentherapie*, **42**: 793.

MAVOR, J. W., AND D. M. DE FOREST, 1924. The relative susceptibility to x-rays of the eggs and sperm of Arbacia. *Proc. Soc. Exper. Biol. and Med.*, **22**: 19.

REGAUD, C., 1924. Some biological aspects of the radiation therapy of cancer. *Am. Jour. Roentgenol.*, **12**: 97.

WATERMAN, N., AND L. DE KROMME, 1927. Ueber den indirekten Faktor in der therapeutischen Strahlenwirkung. *Fortschr. a. d. Geb. d. Röntgenstrahlen*, **35**: 783.

WOOD, F. C., 1925. Immunity in cancer. *Jour. Am. Med. Assn.*, **85**: 1039.

INDIRECT EFFECTS OF RADIATION ON ELODEA LEAVES[1]

L. V. HEILBRUNN AND KATHRYN DAUGHERTY

University of Pennsylvania

In spite of the large literature on the action of radiation on living material, the indirect effects of radiation are but little known. Radiologists and clinicians, interested in the practical use of rays as curative agents, have argued back and forth as to whether or not radiation can act indirectly, and as to how such action if it occurs at all does take place. Some authors have insisted on the existence of so-called necrohormones, which are supposedly produced by certain irradiated cells and then act on other cells more or less distant from them. Unfortunately there is very little experimental evidence which is at all reliable. For references to some of the literature, see Heilbrunn and Young, 1935. The older papers deal mostly with opinions or with experiments on very complicated systems such as are found in higher animals. There is a need for accurate studies on relatively simple types of living matter.

Plant material has certain advantages. It is readily available, and the individual cells are often easy to observe. The leaf of the common water plant *Elodea* is sensitive to radiation (Nadson and Rochline-Gleichgerwicht, 1928), and the effects are readily visible and easily studied. If one exposes half of a leaf, is there any effect observable on the unexposed portion?

We used *Elodea canadensis*. The leaves were subjected to the rays of a Cooper Hewitt lamp of the Uviarc type. We were fortunate to be able to secure such a lamp from the General Electric Company, to whom our thanks are due. The leaves were placed at a distance of 17–19 cm. from the arc.

In view of the fact that we were not primarily interested in the effect of varying wave lengths or varying intensities, we used the unfiltered radiation and made no attempt to calibrate the intensity. Care was taken to exclude heat effects. This was done by the use of two electric fans.

In general, when streaming plant protoplasm is irradiated either with ultraviolet radiation, roentgen rays, or radium, small doses cause an increase in the rate of protoplasmic streaming, whereas larger doses cause decrease in the rate of streaming and eventual stoppage. In the case of *Elodea*, the cells then become filled with calcium oxalate crystals. The doses we used were in most cases large enough to slow or stop movement in the part of the leaf exposed directly, and in all exposures of a minute or more, oxalate crystals formed in the vacuoles of the cells.

---

[1] Aided by a grant from the Committee on Radiation of the National Research Council.

Leaves were cut off an hour or more before they were used in the experiments. A single leaf was then placed on a slide, one half of it was covered with a coverslip and every effort was made to secure the coverslip in such a way that it could not shift position during the experiment. Various devices were tried. The best method was to secure the free end of the leaf by wedging it against a trapezoidal piece of thin celluloid with one side cut to fit approximately the shape of the leaf. This in turn was held in place by a strip of thick celluloid cut to the size of the slide, and with a window which was cut to allow space for the coverslip and the trapezoidal piece of celluloid. This thick celluloid strip was then tied over the glass slide with thread. Once in place it held both coverslip and leaf firmly in position. This could be checked by noting the position of the boundary of the coverslip with reference to definite cells in the leaf.

In general the tip end of the leaf was covered with the coverslip, but in some cases the cut basal end was covered and the tip exposed. The experimental results were essentially the same in either case. During exposure to the radiation, care had to be taken to prevent evaporation of the water surrounding the leaves. This was done by adding water distilled from a fused silica (Kendall) still. Such water in itself has little or no effect on the rate of streaming. Moreover, the distilled water was added both to the experimental leaf and the control.

Following exposure to ultraviolet rays, the leaves half covered with glass were placed under a microscope and observed. For all but the briefest exposures, the cells in the exposed half of the leaf, that is to say the part of the leaf outside of the coverslip, showed complete cessation of protoplasmic streaming. These cells were full of crystals which appeared to be calcium oxalate. In the glass-covered half of the leaf, at least in those cells near the exposed portion, protoplasmic streaming was more rapid than normal. In order to make certain of this point, many determinations of the rate of streaming were made. The rate of protoplasmic streaming varies from cell to cell. In order to be certain of a representative value, at least fifty cells were timed and the average of these cells was recorded. The cells measured were those adjacent to the uncovered half of the leaf, but only those cells completely under the coverslip were observed. The boundary of the coverslip passes over a series of cells which are partly under and partly outside of the cover. The cells directly adjacent to these boundary cells were the cells observed. Thus, on the average, they were at a distance of half the length of a cell from the border of the coverslip.

Although the determination of the rate of protoplasmic streaming of the cells was begun immediately after exposure to the radiation was completed, the measurement of so many cells required approximately twenty minutes. A second series of measurements was begun one hour after the end of the exposure.

The results are shown in Table I. In every case the cells at a little distance from those exposed to ultraviolet show a marked effect —the streaming is decidedly more rapid. Thus when half of an *Elodea* leaf is exposed to the ultraviolet rays for 5 minutes, cells in the unexposed part of the leaf may show an increase in rate of streaming of over 100 per cent. An effect on these cells under the coverslip can be noted even after exposures of only 5 seconds. That this increase in rate of streaming is not due to a direct effect of rays of longer wave length which pass through the coverslip is indicated by the controls in which the whole leaf was covered. In these controls, there is often (though not always) an increase in the rate of streaming, but these increases in the control leaves never really approach those of the experimental leaves. It is true that two of the control leaves showed an exceptionally large increase in rate of streaming. This was due to an increase in room temperatures. In these two cases, the experimental leaves studied at approximately the same time as the controls showed a decidedly greater increase in rate of streaming. So that, on the whole, all of the results are thoroughly consistent and they all indicate a marked indirect effect of the radiation.

In the longer exposures of 15 or 20 minutes there is evidence that the indirect action of radiation may cause not only an increase in the rate of streaming, but also the opposite effect of completely stopping the movement of the protoplasm. Thus in the second of the 15-minute experiments listed in the table, although the rate of streaming was increased in those cells in which streaming was measured, there were 15 cells (out of 50) in which streaming did not occur at all. These cells were not considered in the determination of the rate of protoplasmic streaming. Other experiments with 15-minute exposures gave no such result, but in the case of one of the 20-minute exposures, 8 of the cells (out of 50 observed) showed no streaming. Moreover, in

TABLE I

Indirect effect of ultraviolet radiation on the rate of protoplasmic streaming in *Elodea* cells. Half of leaf covered.

					Control (entire leaf covered with glass and exposed)			
Length of exposure	Rate before exposure	Rate immediately after exposure	Rate 1 hour after exposure	Percentage change after 1 hour	Rate before exposure	Rate immediately after exposure	Rate 1 hour after exposure	Percentage change after 1 hour
	microns/second	*microns/second*	*microns/second*		*microns/second*	*microns/second*	*microns/second*	
5 sec.	15.4	14.1	22.9	+48.6	14.1	14.6	14.6	+3.4
10 sec.	15.1	13.4	22.9	+44.3	15.7	14.6	15.7	0
1 min.	11.7	11.7	13.9	+18.8	12.8	13.0	11.3	−13.2
5 min.	10.1	24.0	25.0	+147.5	14.9	18.4	19.3	+2 .5
5 min.	13.9	19.7	25.0	+79.8	16.0	20.7	18.0	+12.5
10 min.	15.1	22.9	25.7	+70.1	21.2	19.7	19.3*	−9.8
10 min.	15.4	17.0	20.2	+31.1	15.5	16.0	18.0	+16.1
10 min.	10.9	12.5	15.1†	+38.5	14.4	15.1	15.1	+4.8
15 min.	11.4	16.6	22.3*	+95.6	10.4	6.20	15.4*	+48
15 min.	12.5	14.4	18.1	+44.8	24.2	24.2	22.3	−8.5
15 min.	15.1	17.0	22.9	+51.6	14.9	15.1	16.0	+7.3
15 min.	15.7	16.6	19.3	+22.9	17.7	15.1	15.4	−14.9
15 min.	11.3	18.4	20.2	+78.7	17.0	18.1	18.0	+5.8
15 min.	13.7	18.4	20.7	+51.0	16.3	16.6	17.7	+8.5
15 min.	13.4	17	23.6	+76.1	18.4	19.3	19.3	+4.8
15 min.	11.1	15.1	21.6	+94.5	15.4	14.1	15.4	0
15 min.	11.6	17.7	18.4	+58.6	18.8	19.7	19.7	+4.7
20 min.	10.7	17.7	15.7	+46.7	17.0	18.4	17.0	0

* Rate 2 hours after exposure.

† Rate 1½ hours after exposure.

these experiments with longer exposures, not infrequently crystals of calcium oxalate appeared in these cells not directly exposed to the radiation. In some cases fully formed characteristic crystals were observed one hour after the preparations were removed from the ultraviolet. Other cells showed tiny crystals. As time passes, the number of cells with large characteristic crystals increases, and the number of cells with tiny crystals decreases, so that apparently the small crystals are an early stage of the larger ones.

As a whole, the results indicate that when *Elodea* cells are irradiated, substances are produced which can affect neighboring cells. Unfortunately, however, the experiment is not decisive. It might be argued that the cells under the coverslip but near the boundary are directly affected by ultraviolet rays diffracted from the coverslip border. An attempt was made to estimate the extent of such a diffraction effect by photographic tests, but these tests were discontinued in favor of a second and more decisive series of experiments.

Elodea leaves in the water of their culture (tap water) were irradiated, then a non-irradiated leaf or half-leaf was placed in their neighborhood. The non-irradiated leaf then soon showed effects. Protoplasmic streaming was decidedly hastened. The results are shown in Tables II and III. In the experiments recorded in Table II, a half leaf was placed near five previously irradiated leaves. All of the leaves were immersed in a large drop of water on a slide. Another half-leaf served as a control and it was placed in proximity to five non-irradiated leaves. Table III gives the results of a few tests in which leaves attached to the plant were similarly subjected to the influence exerted by irradiated leaves.

Both sets of experiments give the same result. Irradiated leaves have a potent action on other unexposed leaves in their vicinity. There is thus clear and incontrovertible evidence for the indirect effect of this type of radiation.

We believe this indirect action to be due to the diffusion of substances from the irradiated leaves or half-leaves. In view of the remarkable explanations that some authors have postulated as to the possible mechanism of the indirect effects of radiation, it might be argued that there is a secondary radiation or something of the sort which passes out from the exposed part of a leaf. It scarcely seems conceivable that such a secondary radiation could be delayed so as to act after the leaves are no longer exposed to the radiation, and yet even such a theory might find its adherents. To eliminate any such possible explanation, we radiated leaves in culture water and then after the irradiated leaves were removed, a fresh half-leaf was added

TABLE II

Indirect effect of ultraviolet radiation on the rate of protoplasmic streaming in *Elodea* cells. Effect of irradiated leaves on half of an unexposed leaf. Leaves irradiated 20–30 minutes.

					Control I (half of unexposed leaf)				Control II (half of leaf exposed to 5 non-irradiated leaves)			
Number of experiment	Number of rows of cells observed	Rate before exposure to leaves	Rate 1 hour after exposure	Percentage change	Number of rows of cells observed	Rate before exposure	Rate 1 hour after exposure	Percentage change	Number of rows of cells observed	Rate before exposure	Rate 1 hour after exposure	Percentage change
		microns/second	*microns/second*			*microns/second*	*microns/second*			*microns/second*	*microns/second*	
1*	57	13.5	24.6	+82.2	55	17.5	17.7	+1.1				
2	65	10.3	25.1	+143.6	30	12.5	10.8	−15.7				
3	75	12.3	20.7	+60.1	80	12.1	12.3	+1.6				
4	65	20.7	31.5	+52.1					70	16.6	18.0	+8.4
5	50	14.6	24.2	+65.7					—	No	control	—
6	50	14.7	29.3	+99.2					50	17.7	18.0	+1.6
7	85	13.5	27.0	+100.0					75	19.7	19.3	−2.0
8	50	18.8	28.3	+50.5					50	17.7	18.0	+1.6

* 4 leaves used instead of 5.

to the water in which the leaves had been irradiated. With experiments of this type it is possible to show beyond any reasonable doubt that irradiated leaves do actually give off to the surrounding medium substances which have a definite effect on other leaves. As to the nature of these substances, we prefer not to venture any very definite opinions, but to await the result of further experimentation. In view of the fact that radiation of *Elodea* releases calcium (Nadson and Rochline-Gleichgerwicht, 1928), it is possible that this element may be a factor in the interpretation. Perhaps also amino acids play a part (*cf.* Fitting, 1933), or it may be that auximones are concerned.

Whatever the ultimate explanation may be, it is perhaps interesting to note that in these experiments we have another clear case in which stimulated protoplasm produces substances which have an effect on other protoplasm. Examples of this phenomenon are being reported in increasing numbers in all branches of biology, and our knowledge of so-called humoral physiology is rapidly widening. From the standpoint of protoplasmic mechanics and the colloid chemistry of protoplasm, this is a point of great interest. In many ways protoplasm behaves like blood, and the stiffening or gelation of protoplasm is similar to blood clotting (*cf.* Heilbrunn, 1928, 1934). If this parallel be followed, it is easy to understand why stimulation of protoplasm should produce substances which affect resting protoplasm, for stimulated protoplasm can be thought of as similar to clotted blood, and when blood clots, substances are produced which produce clotting in other samples of blood.

TABLE III

Indirect effect of ultraviolet radiation on the rate of protoplasmic streaming in *Elodea* cells. Effect of five irradiated leaves on the streaming of a leaf attached to a plant. Leaves were irradiated 30–40 minutes.

					Control—Leaf on stem not exposed to irradiated leaves			
Number of experiment	Number of rows of cells observed	Rate before exposure to leaves	Rate 1 hour after exposure	Percentage change	Number of rows of cells observed	Rate	Rate 1 hour after first observation	Percentage change
		microns/sec.	*microns/sec.*			*microns/sec.*	*microns/sec.*	
1	75	10.8	16.6	+53.7	70	10.3	12.1	+17.4
2	50	11.1	18.6	+67.5	50	11.4	11.9	+4.3
3	50	9.7	17.0	+75.2	50	10.6	11.3	+6.6

SUMMARY

Cells of *Elodea* leaves exposed to ultraviolet rays produce substances which have an effect on other non-irradiated cells.

LITERATURE CITED

FITTING, H., 1933. Untersuchungen über den Protoplasmaströmung auslösenden Reizstoff der Pflanzen. *Naturwiss.* **21**: 489.

HEILBRUNN, L. V., 1928. The Colloid Chemistry of Protoplasm. Berlin, 1928.

HEILBRUNN, L. V., 1934. The action of anæsthetics on living protoplasm. *Proc. Am. Phil. Soc.*, **74**: 159.

HEILBRUNN, L. V., AND A. R. YOUNG, 1935. Indirect effects of radiation on sea-urchin eggs. *Biol. Bull.*, **69**: 274.

NADSON, G., AND E. ROCHLINE-GLEICHGERWICHT, 1928. Apparition des cristaux d'oxalate de calcium dans les cellules végétales sous l'influence de la radiation ultra-violette. *Compt. Rend. Soc. Biol.*, **98**: 363.

THE MITOTIC FIGURE AND CLEAVAGE PLANE IN THE EGG OF PARECHINUS MICROTUBERCULATUS, AS INFLUENCED BY CENTRIFUGAL FORCE

ETHEL BROWNE HARVEY

(*From the Stazione Zoologica, Naples,*[1] *and the Biological Laboratory, Princeton University*)

The Mitotic Figure

When the eggs, both unfertilized and fertilized, of *Parechinus microtuberculatus*, are centrifuged, the typical stratification is (1) oil at the centripetal pole, (2) yolk granules, (3) clear layer and (4) mitochondria at the heavy pole (E. B. Harvey, 1933). When the eggs are centrifuged after the mitotic figure has formed, this lies (in most batches) among the yolk granules and can be easily observed in the living egg. In the eggs of most other forms, the clear layer lies under the oil cap and the mitotic figure lies in the clear layer; the lack of granules around the mitotic figure prevents its visibility in the living material. Such is the case with the eggs of *Sphærechinus granularis*, *Paracentrotus lividus*, *Arbacia pustulosa* (of Naples), *A. punctulata* (of Woods Hole) and also in some batches of *Parechinus microtuberculatus*, where there is, in addition to the clear layer above the mitochondria, also a clear layer under the oil.

The eggs of *Parechinus microtuberculatus* were centrifuged, as previously described (E. B. Harvey, 1933) in a sucrose solution of the same density and tonicity as the eggs, for 4 minutes with a force of about 7,000 × g. If centrifuged 50 minutes after insemination (19° C.) while still spherical, the mitotic figure is (in many batches) thrown toward the centripetal pole under the oil cap, both in eggs with fertilization membranes and in those from which they have been previously removed (Figs. 1, 2). These eggs do not orient in the centrifuge, and the mitotic figure simply moves to the centripetal pole without shifting its axis. The mitotic figure, therefore, may lie with its long axis in any plane with reference to the stratification (parallel in Fig. 1 and perpendicular in Fig. 2). The spindle, that is, the figure between the asters, is not visible in the living material;

---

[1] I wish to express my sincere thanks to Dr. Reinhard Dohrn, Director of the Stazione Zoologica, for his kindness and interest during my stay in Naples, and my appreciation of the facilities offered there through the Jacques Loeb Memorial Table of the Rockefeller Institute, New York.

the visible figure consists of the two asters. The astrospheres—the clear centers of the asters—tend to elongate in a plane parallel with the stratification. The rays of the asters, as indicated by the granules, are much shortened near the centripetal pole and greatly *elongated toward the centrifugal pole.* This may be observed during rotation, in eggs centrifuged on the centrifuge microscope (with a high power lens which, together with the ocular used, gives a magnification of 400 ×); it may also be observed after rotation in eggs centrifuged in tubes in an ordinary high speed centrifuge and then transferred to watch glasses. The division plane may come in, as indicated in Fig. 2, through the elongate rays. The rays follow somewhat the contour of the cell; this is particularly evident in cells in the centrifuge microscope slide which are indented by particles of débris; the rays follow the indentation. As noted in a previous paper (1934), oil droplets are found entangled in the astral rays, caught there during their passage to the centripetal pole, and particularly in that part of the rays near the astrospheres. Even in eggs, such as those of *Sphærechinus granularis*, where the asters lie in the clear zone, the oil droplets become enmeshed in the invisible astral rays so that these become outlined, especially near the center, by the oil drops (E. B. Harvey, 1934).

In some batches of eggs, the mitotic figure is slightly heavier than the granules and is driven by centrifugal force toward the centrifugal pole, just above the clear layer and mitochondria. In these eggs, the astral rays are greatly *elongated toward the centripetal pole* and shortened or lacking toward the centrifugal pole (Fig. 3). With a greater centrifugal force, the rays are often curved, forming a remarkable spiral-shaped figure (Fig. 4). These spiral-shaped asters remind one of those described as occurring normally in the eggs of some mollusks and annelids and under certain experimental conditions (treatment with phenyl urethane) in sea urchins (Painter, 1916); in these cases they are apparently due to a shifting of materials in the egg. A slight twisting has also been observed in centrifuged eggs of *Crepidula* (Conklin, 1917) and *Cerebratulus* (Morgan, 1910). The rays can also be twisted mechanically by means of a needle (Chambers, 1917).

The foregoing observations confirm the generally accepted view that the mitotic figure is a region of greater gelation since it can be moved through the cell and since it holds the oil drops. The region of gelation consists definitely of distinct rays (asters) around two clear central zones (astrospheres). The observations also show that the rays of the asters are composed of, or are outlined by, granules and that the extent and configuration of the rays, as well as the astrospheres, are greatly modified by centrifugal force.

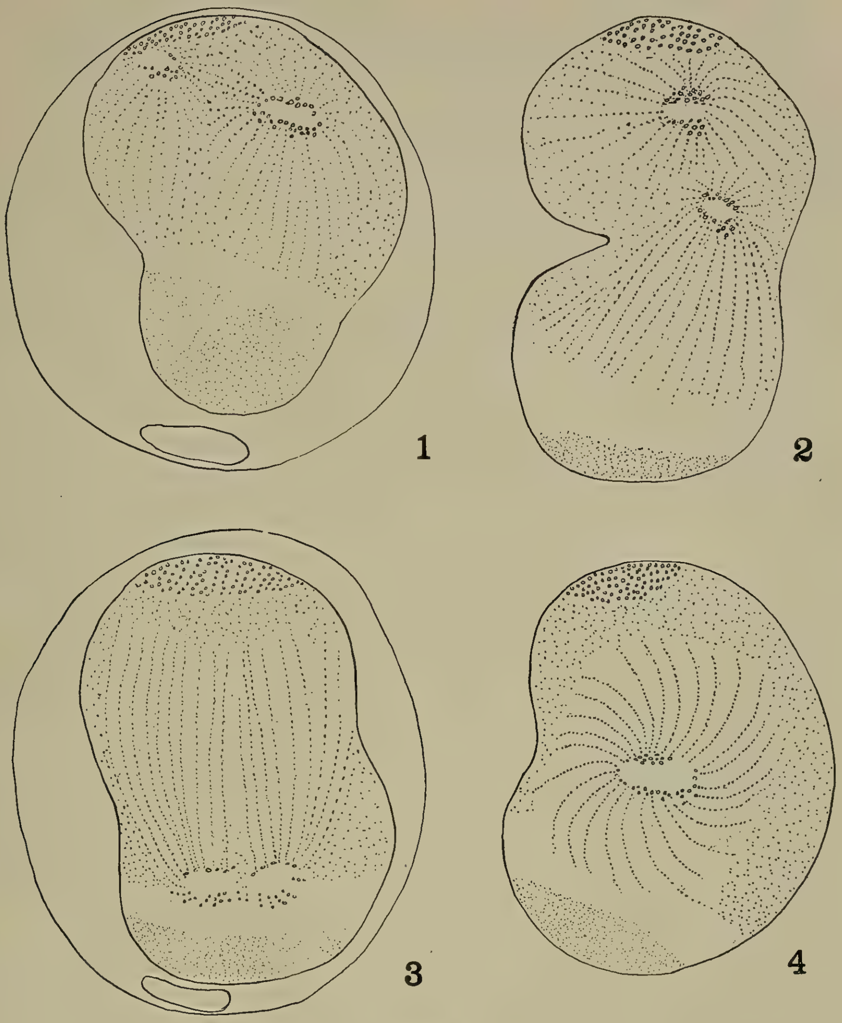


PLATE I

Parechinus microtuberculatus

1. Mitotic figure shifted by centrifugal force to centripetal pole parallel with stratification. Rays shortened toward centripetal pole, elongate toward centrifugal pole. Note oil droplets among rays, also elongation of astrospheres.

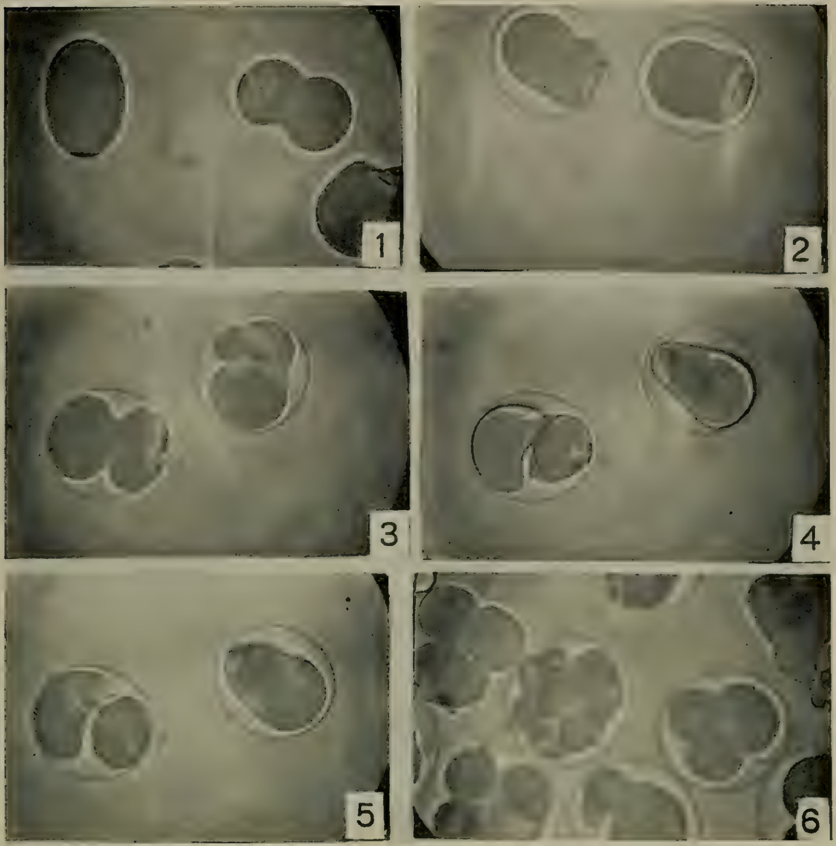
2. Same, mitotic figure in a plane perpendicular with stratification.

3. Mitotic figure shifted to centrifugal pole. Rays shortened toward centrifugal pole, elongate toward centripetal pole.

4. Spiral aster occurring with rapid centrifugal force. Only one aster shown, other one would be behind it.

RELATION OF MITOTIC FIGURE TO CLEAVAGE PLANE

The changes in individual eggs due to centrifugal force can be accurately followed with the centrifuge microscope; the original position of the mitotic figure can be observed, its position after centrifuging, the axis of the egg in relation to centrifugal force, and the cleavage of the egg. The position of the cleavage plane in eggs whose mitotic figure has been displaced is correlated both with the original position of the mitotic figure, which I shall refer to as the "primary" plane, and with its final position, which I shall call the "induced" plane. When a cleavage plane has already started across the equator and the egg lies so that this plane is perpendicular to the centrifugal axis, it may complete itself in spite of the fact that the mitotic figure now lies well above (or below) the equator and bears no relation to the cleavage plane (Photographs 1–6, Fig. 2). It may even cut through the astral rays as noted above (Figs. 2, 3). The plane may also *start* in this position after the eggs have been removed from the centrifuge. The primary plane is usually across the centre of the short axis of the egg; this is especially evident in those eggs from which the fertilization membranes have been removed and which have become somewhat elongate owing to the centrifugal force. This is not necessarily the original plane since, as mentioned above, the eggs do not orient in the centrifuge and one can watch the movement of a mitotic figure whose original position would necessitate a cleavage in a plane perpendicular to the actual cleavage plane (Figs. 1, 3, Photograph 1). In some eggs, particularly in those with fertilization membranes and therefore less elongate, the primary cleavage plane comes in not across the equator, but below, through the clear layer, cutting off the mitochondrial lobe (Photographs 7–10, Fig. 1). This may occur both in eggs whose mitotic figure lay after centrifuging under the oil (Photograph 8) and in those where it lay just above the clear layer (Photograph 9). The mitochondrial lobe which is thus cut off resembles the polar lobe characteristic of some annelid and mollusk eggs. The lobe lacks a nucleus and does not cleave further; the other part of the egg, containing the mitotic figure and subsequently the nuclei, cleaves fairly regularly and forms a blastula (Photograph 10). Having found that the cleavage plane may come in in a position without relation to the final position of the mitotic figure, the question arose as to how far advanced in the cleavage cycle the nucleus must be in order that a primary plane might appear. It was found that the nuclear wall may be still unbroken, and yet the (primary) cleavage plane come in in the region where the nucleus was before centrifuging. Such an



PHOTOGRAPHS 1–6. *Parechinus microtuberculatus*

(The temperature in the experiments was 18–19° C.)

1. Fertilized at 11:35; centrifuged from 12:32–36; photograph taken at 12:38. Cell to right cleaving across the equator though the mitotic figure lies under the oil ("primary" plane). Cell to left cleaved later through the oil perpendicular to the stratification ("induced" plane).

2. Fertilized at 9:10; centrifuged 10:10–10:14; photograph taken at 10:16. Both planes, primary and induced in both eggs.

3. Same eggs, photographed at 10:21. Both cleavage planes more marked in both eggs.

4. Same eggs, photographed at 10:30. Primary cleavage plane obliterated in egg to right; induced plane present but does not show in photograph as it lies in the plane of the paper. Both planes present in egg to left.

5. Same eggs, photographed at 10:35. Induced plane (through oil) still present in egg to right, not well shown in photograph. Primary plane still present in egg to left, induced plane obliterated.

6. Another set of eggs, later stage. Fertilized at 10:13; centrifuged from 11:15–11:19. Photograph taken at 1:10. The cell containing the nucleus has cleaved, the cell lacking it has not cleaved.

egg is shown in Photograph 11. The eggs were centrifuged (for 4 minutes) while in the streak stage; this was 20 minutes before cleavage and 10 minutes before the nuclear wall broke in the controls. It should be noted, of course, that this amount of centrifuging in these eggs at this stage causes only a slight elongation but is not nearly sufficient to break them apart even when they lack fertilization membranes. There is, therefore, no danger of confusion of a cleavage plane with a break caused by centrifugal force. Forty minutes after removal from the centrifuge, the nuclear wall was still intact in this particular egg and yet the cleavage plane had come in across the equator with no relation to the final position of the nucleus (Photograph 11). Twenty minutes later, the nuclear membrane had broken and the mitotic figure had formed; the primary cleavage plane was now much less distinct. Ten minutes later, the primary plane had almost disappeared and a new plane (the "induced") had come in through the mitotic figure (Photograph 12). In this and similar eggs, therefore, a primary cleavage plane may come in through the equator even though the nucleus lies after centrifuging near one pole and while the nuclear wall is still intact.

As stated previously, there is usually a cleavage plane not only through or near the equator of the egg without reference to the new position of the mitotic figure (primary plane), but also a cleavage plane correlated with the new position of the mitotic figure, no matter

---

Photographs 7–14. *Parechinus microtuberculatus*

7. Egg centrifuged just before cleavage. Cleavage plane indicated just above mitochondrial lobe.

8. Fertilized at 10:15; centrifuged from 11:15–11:19; photograph taken at 11:35. Cleavage above mitochondrial lobe (primary cleavage). Induced cleavage beginning below though mitotic figure is above, near the oil.

9. Fertilized at 10:05; centrifuged from 11:08–11:12; photograph taken at 11:35. Cleavage above mitochondrial lobe. Induced cleavage starting below near mitotic figure and lagging at oil cap.

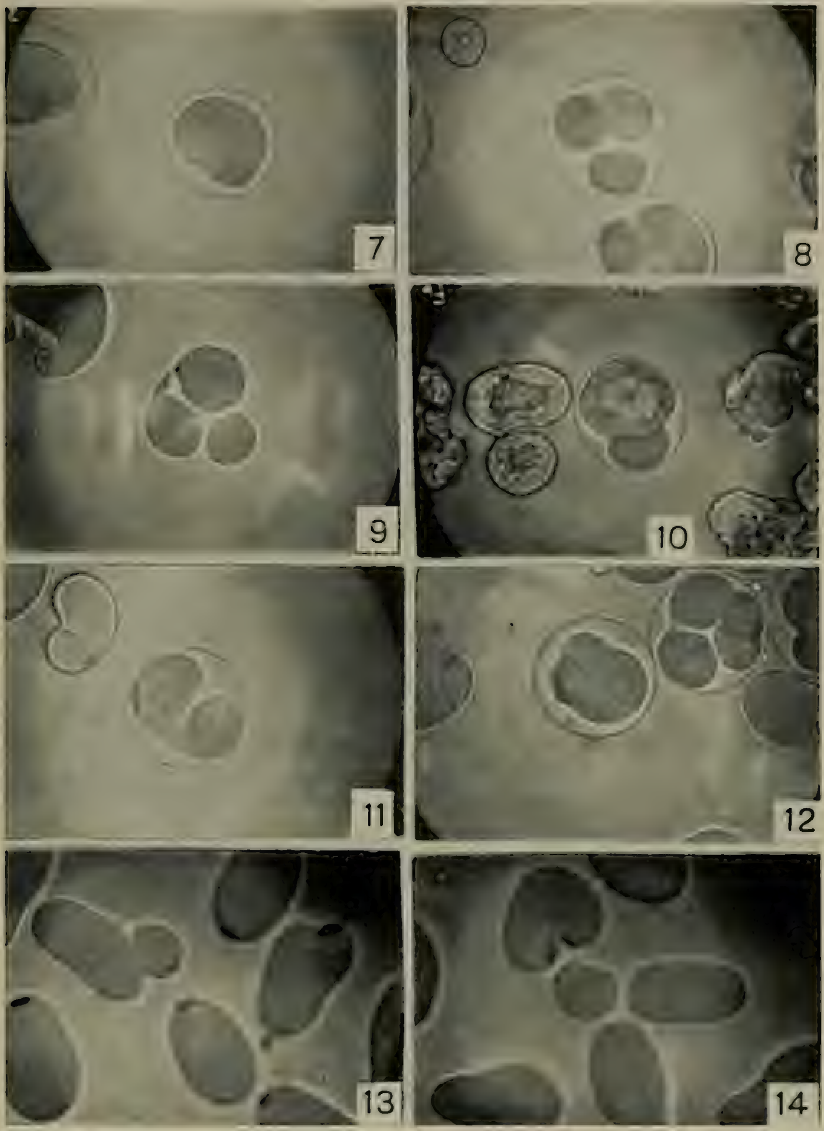
10. Fertilized at 9:40; centrifuged from 10:40–10:44; photograph taken at 4:22. Central egg—upper cell having nucleus has developed into blastula, lower cell (mitochondrial lobe) lacking nucleus, has not developed. Egg to left had nucleus in both cells, and both have developed into blastulae.

11. Fertilized at 11:15; centrifuged from 12–12:04; photograph taken at 12:45. Cleavage (primary) though nucleus still intact (in upper part). Nuclear wall in controls broke at 12:10, cleavage at 12:20.

12. Same egg at 1:17. New induced plane across mitotic figure which formed at 1:05—primary plane almost obliterated.

13. Fertilized at 10:00; centrifuged from 10:43–10:47; photograph taken at 10:55. Cleavage in controls at 11:05. Induced cleavage before cleavage in controls.

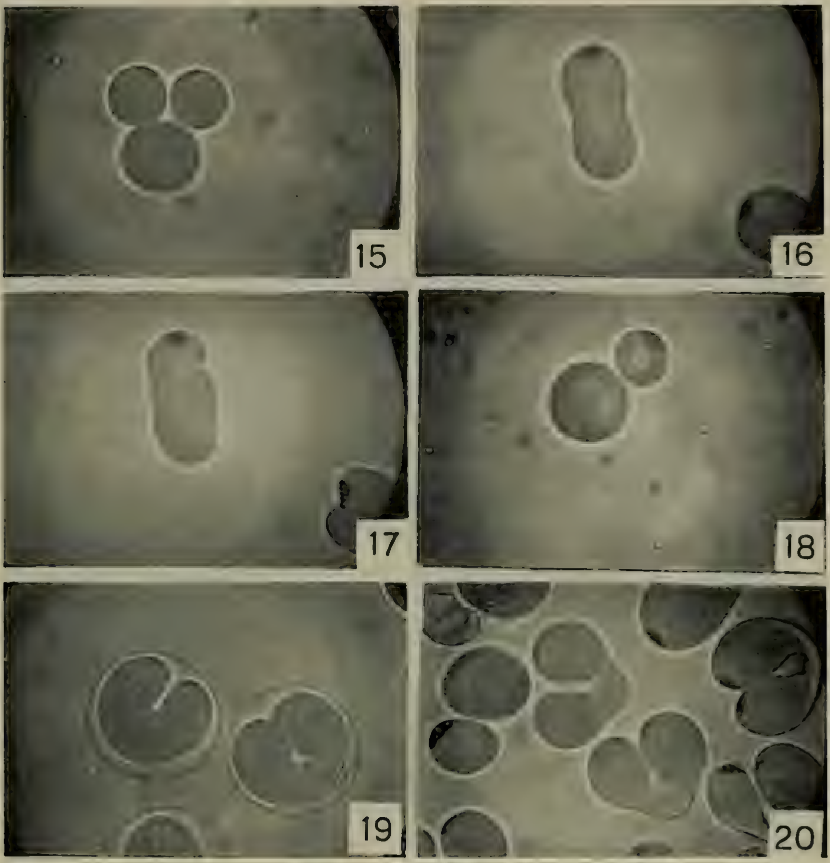
14. Another egg from same set at 11:04; induced cleavage complete in central egg; oil cap in cell to left.


7
8
9
10
11
12
13
14

Photographs 7–14. *Parechinus microtuberculatus* where it lies after centrifuging; this I call the "induced" plane. In those eggs where the mitotic figure is thrown near the oil cap, the induced plane usually comes in here (Photograph 13). In an elongate membraneless egg, this often results in the pinching off of a small piece, as shown in this photograph. If the mitotic figure lies further down, especially in an elongate egg, the cleavage plane usually comes in here. (Photograph 14, central egg which has already cleaved; oil cap to the left.) When the mitotic figure is thrown quite near the surface, the induced plane comes in immediately, sometimes 10 minutes before it does in the normal control eggs (Photograph 13). Frequently both planes, the primary and induced, come in synchronously, or either one may come in before the other (Photographs 2–5). Either or both planes may become functional. In the egg to the left in Photographs 2–5, the primary plane became functional and the induced plane became obliterated; in the egg to the right the reverse took place. In Photograph 15, both planes became functional. In eggs such as these, the cell lacking the mitotic figure and nucleus does not develop while the cell with the mitotic figure cleaves fairly regularly and forms a blastula (Photographs 6 and 10). In some eggs the spindle lies in the long axis after centrifuging, and after a cleavage in the primary plane, an induced plane may also come in *parallel* to it (Photographs 16–18, same egg); in this egg only the induced plane became functional.

The induced plane in these eggs usually starts at the surface nearest the mitotic figure; when this is near the oil cap, the cleavage plane starts here and seems to penetrate the mitochondrial zone with difficulty (Photograph 20); when the mitotic figure is nearest the heavy end of the cell, the cleavage plane usually starts here and lags at the oil cap. (Photograph 9.) It is then not entirely a matter of the density of the material that determines the ease of penetration of the cleavage plane, though this is usually the case. On the other hand, it is not always the nearness of the surface to the mitotic figure that determines its inception, since in some few cases the spindle lies near the oil cap and induced cleavage begins at the heavy end of the cell. (Photograph 8.) In this egg, the mitochondrial lobe has been separated off by the primary cleavage, and the plane of the induced cleavage has started in this zone, even though the mitotic figure lies far removed under the oil cap.

When eggs are centrifuged soon after fertilization, in the monaster or early streak stage and then removed from the centrifuge, the nucleus returns to its central position before the mitotic figure forms. Cleavage is usually equatorial, but without reference to the stratification, as is indicated by the fact that it bears no relation to the oil cap; it possibly bears a relation to the original polarity of the egg before



Photographs 15–20. *Parechinus microtuberculatus*

15. Fertilized at 9:40; centrifuged from 10:40–10:44; photograph taken at 11:30. Both primary and induced planes functional; lower cell has no nucleus.

16. Fertilized at 3:10; centrifuged from 3:48–3:52; photograph taken at 4:20. Cleavage in primary plane; amphiaster above in long axis of egg and its axis perpendicular to primary cleavage plane.

17. Same egg at 4:23; new induced cleavage plane, primary plane nearly gone.

18. Same egg at 4:55; induced plane functional.

19. Fertilized at 10:15; centrifuged from 11–11:04, as nuclear wall is breaking; photograph taken at 11:20. Cleavage through oil cap.

20. Fertilized at 10:00; centrifuged from 10:43–10:47; photograph taken at 11:18; lag of cleavage plane at mitochondrial lobe.

centrifuging, though this was not determined. This is at variance with the eggs of other species, *Paracentrotus*, *Sphærechinus*, and *Arbacia*, where the nucleus lies after centrifuging in the clear zone;

the mitotic figure forms here and the cleavage plane comes in through the oil and perpendicular to the stratification. The discrepancy is probably due to the density of the nucleus (and mitotic figure) relative to the liquid matrix (clear layer) and the granules. In *Parechinus*, the nucleus is (in most batches) lighter than the matrix and of the same weight as the granules. In the other eggs, it is of the same weight as the matrix and lighter than the granules, and its position of equilibrium is in the clear layer, and the mitotic figure forms here with its long axis parallel with the long axis of the layer itself.

If the eggs of *Parechinus* are centrifuged soon before the mitotic figure forms and while the nuclear wall is still intact, the nucleus does not have time to return to the center before cleavage; the spindle usually forms parallel with the stratification and the cleavage plane tends to come in through the oil (Photograph 19). As has been noted above, however, a primary plane may come in, in eggs centrifuged at this stage, bearing no relation to the final position of the nucleus (Photograph 11).

Discussion

The foregoing experiments show that any theory of mitosis based on the mechanical pull of the astral rays on the surface of the cell is inadequate, since cleavage may take place without relation to the final position of the rays. Previous experiments (1934) have shown that any theory involving the ectoplasmic layer as an active agent in cleavage is inadequate since this layer may be centrifuged off from the egg and yet the egg may cleave. It has been shown by the above experiments that the cleavage plane is correlated with the position of the nucleus or mitotic figure both before and after shifting by centrifugal force. Another factor influencing the position of the cleavage plane is the shape of the egg, since it tends to cut across the short axis. Other factors involved are the distribution and density of the moveable materials in the egg, and the time interval elapsing between the change in position of the nucleus or mitotic figure and the occurrence of cleavage. Since a cleavage plane can come in without relation to the mitotic figure, it would seem that the mitotic figure itself is an expression of more fundamental physical and chemical changes taking place in the cytoplasm and surface of the egg, and not a cause in itself of the cleavage process.

Summary

1. The mitotic figure of *Parechinus microtuberculatus* may be shifted by centrifugal force to the centripetal pole, and the astral rays then become much elongate toward the centrifugal pole. In

other batches of eggs it is thrown to the centrifugal pole and the astral rays then become elongate toward the centripetal pole.

2. The first cleavage plane is correlated with the position of the nucleus or mitotic figure before centrifuging, and may bear no relation to its final position (primary plane). It is also correlated with the position of the nucleus or mitotic figure after centrifuging, since another cleavage plane may appear in relation to the new position of the figure (induced plane).

LITERATURE CITED

Chambers, R., 1917. Microdissection studies. II. The cell aster; a reversible gelation phenomenon. *Jour. Exper. Zoöl.*, **23**: 483.

Conklin, E. G., 1917. Effects of centrifugal force on the structure and development of the eggs of Crepidula. *Jour. Exper. Zoöl.*, **22**: 311.

Harvey, E. B., 1933. Development of the parts of sea urchin eggs separated by centrifugal force. *Biol. Bull.*, **64**: 125.

Harvey, E. B., 1934. Effects of centrifugal force on the ectoplasmic layer and nuclei of fertilized sea urchin eggs. *Biol. Bull.*, **66**: 228.

Morgan, T. H., 1910. Cytological studies of centrifuged eggs. *Jour. Exper. Zoöl.*, **9**: 593.

Painter, T. S., 1916. Contributions to the study of cell mechanics. I. Spiral asters. *Jour. Exper. Zoöl.*, **20**: 509.

SOME SURFACE PHENOMENA IN THE FERTILIZED SEA URCHIN EGG AS INFLUENCED BY CENTRIFUGAL FORCE

ETHEL BROWNE HARVEY

(*From the Stazione Zoologica, Naples, and the Biological Laboratory, Princeton University*)

STREAK STAGE

There is a certain period during the development of the normal egg of *Parechinus microtuberculatus* during which the surface becomes crenate. The eggs from some females show the phenomenon markedly and the eggs from others not at all. The crenation occurs during the streak stage—that is, the period between the end of the monaster (20 minutes after fertilization at 19° C.) and the formation of the mitotic figure (45 minutes after fertilization and 15 minutes before cleavage). As soon as the nuclear wall breaks and the mitotic figure forms, the eggs become perfectly spherical again (Photographs 1 and 2, same eggs). The change in the condition of the protoplasm caused by the breakdown of the nuclear wall is no doubt correlated with this latter surface change. This crenation phenomenon was observed and noted previously during some experiments with lack of oxygen, which accentuates it (E. B. Harvey, 1927). Although the crenation resembles that due to hypertonic sea water, it obviously cannot be due to this, since the same eggs in the same sea water later become spherical. Hoadley (1934) has noted "pulsations," or rhythmic changes in shape of the *Nereis* egg which are somewhat comparable with those occurring in *Parechinus*.

When the eggs, with fertilization membranes intact, are centrifuged at this time (5,000 × g for 4 minutes in an isotonic sucrose solution and then removed to sea water), the part of the egg toward the centrifugal pole is thrown into folds while the part toward the centripetal pole remains smooth (Photograph 3). This occurs in all batches of eggs, even in those which show no crenation under normal conditions during this period. At the time that the nuclear membrane breaks in these eggs the folding disappears, the entire surface of the egg becomes quite smooth, and the egg is again spherical (Photograph 4, same eggs as shown in Photograph 3).

If the eggs are centrifuged earlier, during the monaster stage, the folding does not take place at the time of centrifuging. As soon, however, as they reach the streak stage, a change takes place in the surface of these eggs, and they become sometimes quite like those centrifuged during the streak stage and sometimes crenate all over the surface like the controls, depending upon the time elapsing between centrifuging and the normal crenation period. If they are centrifuged just before the time for crenation, the centrifugal surface subsequently becomes folded; if centrifuged somewhat earlier, the whole surface later becomes crenate. If the eggs are centrifuged after the normal crenation period, that is, after the mitotic figure has formed, no folding takes place. Photograph 5 shows two eggs centrifuged at the same time, the one to the left in the streak stage, with nucleus still intact, and the one to the right in the spindle stage; the former is decidedly folded, the latter spherical.

If placed in hypotonic sea water, both the normally crenate eggs and the folded centrifuged eggs become spherical. The crenation is, therefore, influenced by osmotic conditions.

When normal eggs have been freed of their fertilization membranes by shaking soon after insemination, they acquire the same crenate surface at the streak stage as those with fertilization membranes. When membraneless eggs are centrifuged during the streak stage, the surface of the elongated egg toward the centrifugal pole becomes rough and crinkled while the surface toward the centripetal axis remains smooth (Photographs 6 and 7).

The eggs of *Paracentrotus lividus* and *Sphærechinus granularis* show the same phenomenon as those of *Parechinus microtuberculatus* when centrifuged at the streak stage, but in a less degree.

When *unfertilized* eggs (*Sphærechinus*) are centrifuged and put into hypertonic sea water, a crenation of the surface takes place, but chiefly near the centripetal pole (Photograph 8). The hypertonic sea water causes artificial parthenogenesis in many eggs. As soon as the (fertilization) membrane is thrown off, the surface immediately becomes smooth (Photograph 8), an evidence of marked surface changes which are known to be occurring at the time of the elevation of the membrane.

Significant observable changes, therefore, take place in the surface of the egg during development, and these changes are constant for particular stages. It has been shown previously (1933) that there are characteristic differences in the mode of breaking of unfertilized and fertilized eggs, and of the fertilized eggs at different periods after fertilization when they are subjected to a strong centrifugal force; and these differences have been attributed to surface changes. It seems possible that these surface changes demonstrable both in normal eggs and in those subjected to centrifugal force are correlated with changes in permeability. It is well known that the fertilized egg is much more permeable to water than the unfertilized (R. S. Lillie, 1916; McCutcheon and Lucké, 1932), and Lillie (1918) has shown that the permeability of the fertilized egg varies with the stage after fertilization. It becomes marked 20 minutes after fertilization in the *Arbacia* egg at 20° and reaches a maximum somewhat later; at this time this egg is in the streak stage. The marked changes in permeability therefore occur at the same stages as the marked observable changes in the surface.

Two-cell Stage

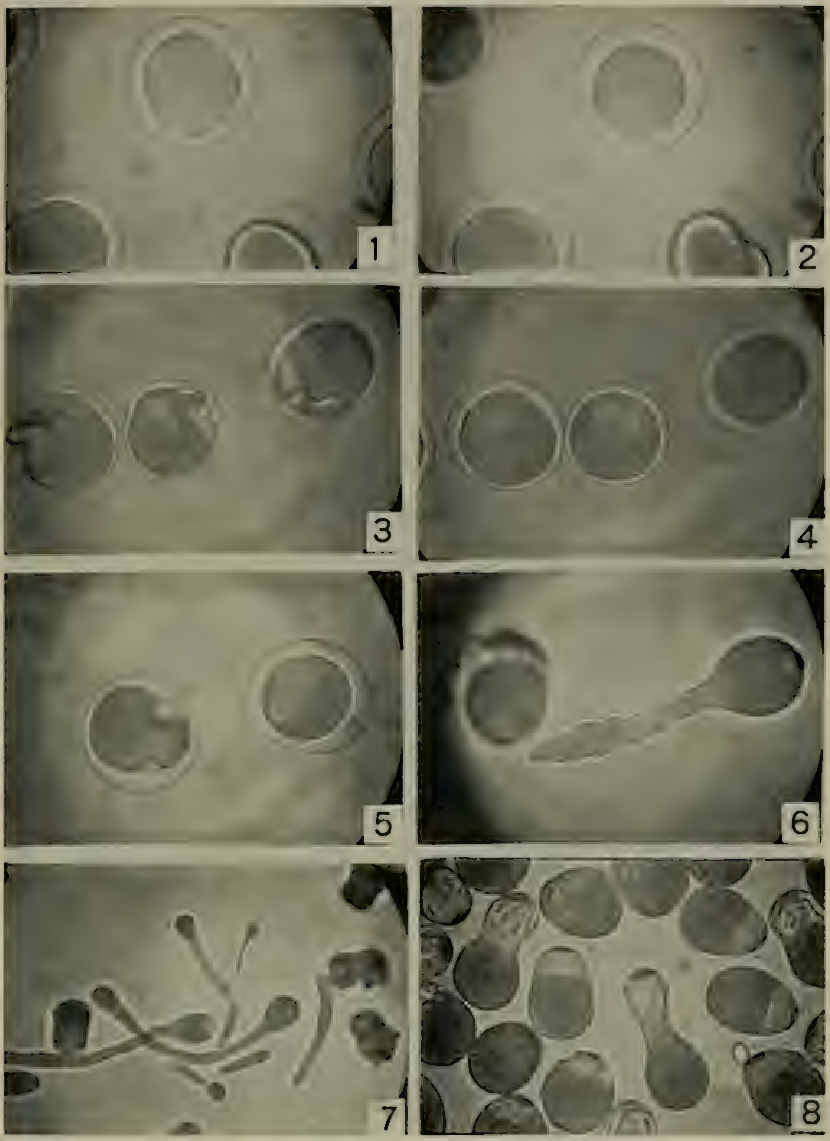
When the egg of *Parechinus microtuberculatus* is centrifuged (5,000 × g for 4 minutes in an isotonic sucrose solution) in the 2-cell stage, each of the two cells stratifies in the same way as the single cell. The ectoplasmic layer is, as described in a former paper (1934) thrown off by the centrifugal force. This causes the cells to separate since the binding material is lacking. After centrifuging, the eggs were returned to sea water. The cells without membranes (membranes removed immediately after fertilization by shaking) have become greatly elongate and the narrow tails are often intertwined (Photographs 9 and 10). The two cells are often quite widely separated, both those with and those without membranes. After a little while,

---

Photographs 1–8. Crenation.

(1–7 *Parechinus microtuberculatus;* 8. *Sphærechinus granularis.* Temperature 18–19° C.)

1. Normal egg; fertilized at 11:37; photographed at 12:20. Streak stage showing crenation; nucleus still intact.
2. Same egg at 12:25 after formation of mitotic figure; surface spherical.
3. Eggs fertilized at 10:37; centrifuged from 11:08–11:12 at streak stage; photographed at 11:20. Surface folded at centrifugal pole.
4. Same eggs at 11:35 after formation of mitotic figure. Surface smooth, folding has disappeared.
5. Eggs fertilized at 11:35; centrifuged from 12:20–12:24. Egg to left with nucleus intact showing folding of surface; egg to right after nuclear membrane has broken showing smooth surface.
6. Fertilized at 12:15; centrifuged from 12:45–12:49 at streak stage, fertilization membrane having been previously removed; surface of egg crinkled at centrifugal end. An egg at left, out of focus, with membrane intact showing folding as in Photograph 3.
7. Fertilized at 11:15; centrifuged from 12–12:04; photographed at 12:08 at streak stage. Eggs without membranes have crinkled tails, those in membranes folded.
8. *Sphærechinus granularis.* Unfertilized eggs centrifuged, then put into hypertonic sea water. Show crenation at centripetal pole. Some have thrown off membranes, developing parthenogenetically. Note smooth surface of these.



Photographs 1–8. Crenation.

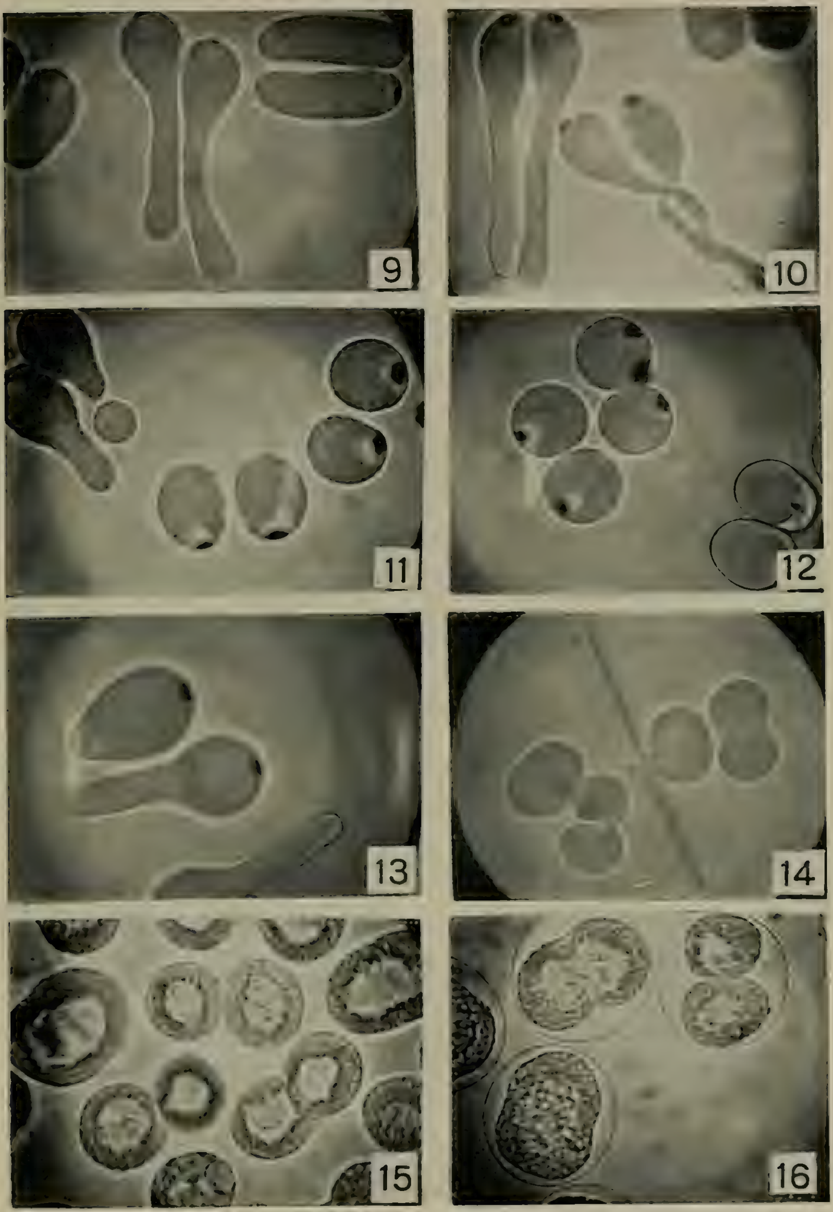
however, the two cells may approach each other and come again into contact. One can often see papillæ form on the adjacent surfaces of the two cells and later threads stretch between, pulling the two cells close together (Photographs 11, 12). These cells then divide and later form a single normal blastula. A similar binding process has been observed between two whole intact eggs after centrifuging (Photograph 13), but the threads broke later on and the two cells separated. The knitting process is doubtless due to the regeneration of the ectoplasmic layer. It has been noted previously (1934) that this layer *does* regenerate after having been removed. These observations give additional evidence that the function of the layer is to act as a binding material holding together individual cells. Similar observations on the coalescence of artificially isolated cells have been made by Hammar (1896), H. V. Wilson (1911), and others. Recently A. R. and M. M. Moore (1931) have called attention to the importance of such strands or "cell-bridges" in the developing sea urchin egg.

Frequently the first two blastomeres which have been separated by centrifuging, remain apart and develop separately into twin blastulæ (Photographs 14–16). Under what conditions this occurs, I did not determine, but it is probably due to the failure of the ectoplasmic layer to regenerate. Often the first cleavage is not synchronous in the two cells (Photograph 14). If the fertilization membrane has not been removed, the twins develop into blastulæ inside the membrane, and frequently one swims out much in advance of the other. There are all gradations between the development of twin blastulæ from the first two blastomeres, and the development of one perfect blastula, all degrees of fusion occurring (Photographs 15, 16).

PHOTOGRAPHS 9–16. Two-cell stage.

(*Parechinus microtuberculatus*. Temperature 18–19° C.)

9. Eggs fertilized at 10; centrifuged from 11:25–11:29 in 2-cell stage; fertilization membranes previously removed.
10. Eggs fertilized at 2:55; centrifuged at 4:00; shows intertwining of tails.
11. Eggs fertilized at 3:25; centrifuged at 4:25; photographed at 4:40. Note papillæ and strands between adjacent surfaces of the two blastomeres.
12. Same eggs after 10 minutes, showing contact of blastomeres.
13. Fertilized at 9:40; centrifuged from 10:10–10:14; photographed at 10:25. Two whole cells joined by filaments.
14. Fertilized at 10:48; centrifuged from 12:05–12:08; photographed at 12:45. Note cleavage of blastomeres in both eggs, asynchronously.
15. Fertilized at 9:55; centrifuged from 11–11:04; photographed at 6:15, when just beginning to swim. Twin and partially fused blastulæ.
16. Same with fertilization membranes not removed.


9
10
11
12
13
14
15
16

Photographs 9–16. Two-cell stage.

Summary

1. Some normal *Parechinus* eggs become crenate during the streak stage. When centrifuged at this stage, the cells with fertilization membranes become folded and those without fertilization membranes become crinkled near the centrifugal pole.

2. When centrifuged in the 2-cell stage, the two blastomeres are separated owing to the destruction of the ectoplasmic layer. They may later be drawn together by fine strands, forming a single blastula; or they may remain separate forming twin blastulæ.

LITERATURE CITED

Hammar, J. A., 1896. Ueber einen primären Zusammenhang zwischen den Furchungszellen des Seeigeleies. *Arch. mikr. Anat.*, **47**: 14.

Harvey, E. B., 1927. The effect of lack of oxygen on sea urchin eggs. *Biol. Bull.*, **52**: 147.

Harvey, E. B., 1933. Effects of centrifugal force on fertilized eggs of Arbacia punctulata as observed with the centrifuge microscope. *Biol. Bull.*, **65**: 389.

Harvey, E. B., 1934. Effects of centrifugal force on the ectoplasmic layer and nuclei of fertilized sea urchin eggs. *Biol. Bull.*, **66**: 228.

Hoadley, L., 1934. Pulsations in the Nereis egg. *Biol. Bull.*, **67**: 484.

Lillie, R. S., 1916. Increase of permeability to water following normal and artificial activation in sea urchin eggs. *Am. Jour. Physiol.*, **40**: 249.

Lillie, R. S., 1918. Comparative permeability of fertilized and unfertilized eggs to water. *Science*, **47**: 147.

McCutcheon, M., and B. Lucké, 1932. The effect of temperature on permeability to water of resting and of activated cells (unfertilized and fertilized eggs of Arbacia punctulata). *Jour. Cell. and Comp. Physiol.*, **2**: 11.

Moore, A. R., and M. M., 1931. Fertilization and development without membrane formation in the egg of the sea urchin (Paracentrotus lividus). *Arch. de Biol.*, **42**: 375.

Wilson, H. V., 1911. On the behavior of the dissociated cells in hydroids, Alcyonaria and Asterias. *Jour. Exper. Zoöl.*, **11**: 281.

THE PHOTOTROPIC RESPONSES OF AVENA IN RELATION TO INTENSITY AND WAVE-LENGTH [1]

CHARLES HAIG

(*From the Laboratory of Biophysics, Columbia University, New York City*)

Darwin's (1880) conclusion that the photoreceptors of grass seedlings (with the possible exception of oats) are practically confined to the first few millimeters or tip of the shoot was in the main confirmed by Rothert's (1896) work. Rothert, however, clearly showed that the basal portion of unilaterally illuminated oat coleoptiles whose tips are shielded from the light undergo positive curvature. More recently Bakker has found this to be true also of *Panicum* (F. A. F. C. Went, 1924).

The enormously higher sensitivity of the tip to photic stimulation (Sierp and Seybold, 1926) undoubtedly accounts for the greater attention commanded by this region until F. W. Went (1925, 1926, 1928) showed that in addition to this difference in sensitivity there are striking differences in the responses when the tip and base are stimulated separately. Among other things he found (1925) that an oat plant receiving a general illumination of 500 meter-candle-seconds shows two periods of growth rate inhibition, one beginning about 28 minutes and the other about 8 minutes after stimulation. When all but the upper 1.25 mm. of coleoptile is shielded from the light, only the 28-minute response occurs, and when the upper 3 mm. is shielded, only the 8-minute response occurs. He names these respectively the tip response and the base response.

The present work was undertaken to secure precise measurements of the bending responses of *Avena*, when naked and when shielded, to lights of different intensities and spectral composition, in the hope that these findings would be confirmed and specified.

PROCEDURE

Avena sativa seeds were germinated on moist filter paper placed in the bottom of covered jars. The germinating seeds were kept in a light-proof incubator at 25° C. for about 70 hours, when the roots had attained a length of 20–25 mm., and coleoptiles had not yet appeared. The seeds were then mounted in small vials, containing distilled water, by inserting them into holes bored in the center of the corks. Since all the holes were of the same diameter, all seeds that would slide in with reasonable ease and remain fixed were of about the same size. The mounted seedlings were replaced in the incubator and in 12 hours the coleoptiles were ready for use in an experiment (Fig. 1, *b*). Although preliminary experiments indicated that considerable variation in length did not increase variability in the measurements, only those shoots that were straight and between 8 and 12 mm. long were used.

---

[1] Two preliminary reports on this work have appeared (Haig, 1934*a*, *b*).

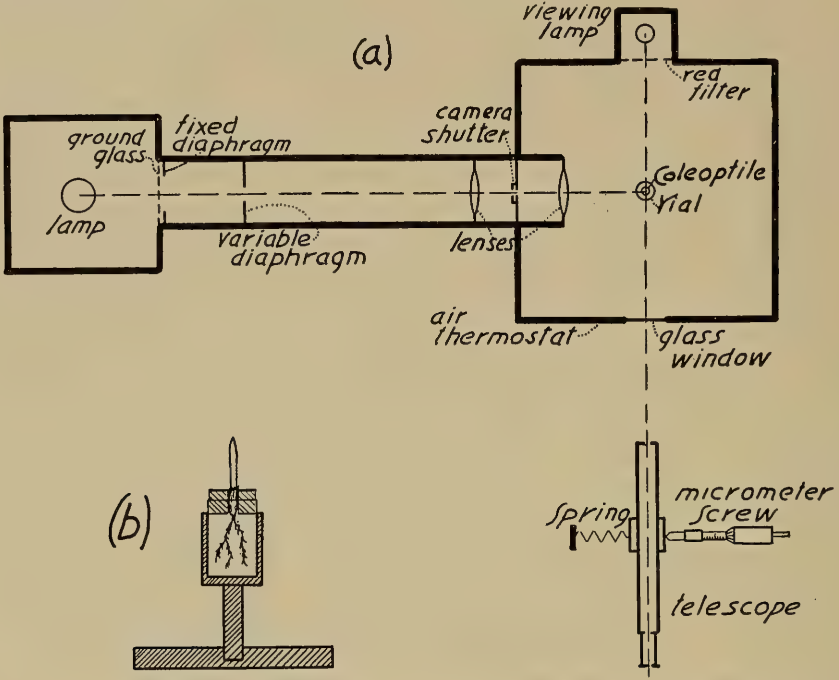

(a)
viewing lamp
red filter
camera shutter
fixed diaphragm
ground glass
coleoptile
vial
lamp
variable diaphragm
lenses
air thermostat
glass window
(b)
spring
micrometer screw
telescope

FIG. 1. (*a*) Plan of apparatus for unilateral illumination of *Avena* by white light. Wratten filters were mounted between the plant and the first lens in the experiments with colored lights. For obtaining spectral lights, the lenses, diaphragms, and concentrated filament lamp were replaced by a neutral wedge, monochromator, and ribbon filament lamp. (*b*) Sectional view of mounted seedling.

During each experiment the plant was kept upright in the center of a thermostatically controlled light-proof box at 25° C. (Fig. 1, *a*). It was so placed that the flat side of the coleoptile was presented to the stimulating light.[2] The position of the shoot apex was followed by the telescope which was displaced horizontally by a micrometer screw. A record of the position was made every minute beginning a few minutes before exposure and continuing well beyond the time when bending had become established. The positions were then plotted against time and the moment for onset of bending was determined graphically to the nearest minute. The time between beginning of exposure and initiation of bending is here called the reaction time.

---

[2] The coleoptile being ellipsoidal in cross-section, the angle formed by the sides of the apex is sharper when viewed in the short than in the long diameter. Accordingly, the flat side was presented to the stimulating light and the vertical hair of the viewing telescope focused on the sharp apex of the narrow side. This practice had the further advantage that any difference in response arising from the plant's asymmetry could not complicate the results.

Since *Avena* has been shown by Blaauw (1909) and others to be quite insensitive to red light, a dim light passed through an Eastman No. 71a Wratten red filter was used for examining and handling experimental plants, and for making observations. Hence no effective stimulus reached them before or after the experimental stimulus was given.

The stimulating source was a 500-watt concentrated filament tungsten lamp located in a separate compartment which communicated with the plant compartment by a wooden tube containing, in the order named, a ground glass plate with brass diaphragm, a fixed diaphragm with attached channels for accommodation of a series of replaceable fixed diaphragms of various diameters, a pair of biconvex lenses, and between them a camera shutter. The lenses were so placed that the emergent beam of light was a parallel bundle. The replaceable diaphragms controlled the intensity at the plant over the range 1-10,000 meter-candles. Exposures of one second were made by means of the camera shutter. Longer exposures were controlled by a stop-watch, using the bulb mechanism of the camera shutter.

In the experiments with colored lights, channels were provided to accommodate Wratten filters (Eastman Kodak Company "Monochromatic") in the path of the light beam. For violet light filter No. 76 (center of gravity at λ4475) was used, and for blue-green light No. 75 (center of gravity at λ4905). In the course of the work it became apparent that the plant was so insensitive to wave-lengths longer than those transmitted by filter No. 75 that only a minus-blue filter (No. 12), which transmits all wave-lengths longer than 5000 Å, could be used without substituting a more powerful source. The energy distribution of the tungsten lamp employed was computed in steps of 100 Å from Wien's equation, using the color temperature 3200° K. (Forsythe, 1920). Each filter was calibrated for percentage transmission at intervals of 100 Å spectrophotometrically and the values so obtained multipled by the energy values for the lamp at these wave-lengths. The resulting transmission curves were plotted and their areas measured with a planimeter. These areas were proportional to the energies incident at the plant when the respective filters were in use.

For later experiments with monochromatic light a monochromator was used with a vertical ribbon filament lamp operating at 18 amperes and 6 volts. In this case the plant was so placed that a narrow colored image of the slit fell on it. A "neutral" gelatine wedge controlled the intensity of the emergent beam. The lamp, lens, and monochromator were calibrated together for energy distribution by photometric comparison with a calibrated monochromator and source. The wedge transmission was calibrated spectrophotometrically for each wave-length used. Thus, for any given wave-length and wedge setting, the relative incident energy at the plant was the product of the relative energy of light leaving the monochromator and the wedge transmission for that wave-length and setting.

TABLE I

Reaction time to white light of various intensities. Average of 10 readings, one on each of 10 plants. Exposure time 1 second.

Coleoptile as a whole				Tip exposed			Base exposed		
Intensity	Average reaction time	Probable error	$\frac{1}{(r-m)}$	Intensity	Average reaction time	Probable error	Intensity	Average reaction time	Probable error
log meter-candles	*minutes*	*minutes*		*log meter-candles*	*minutes*	*minutes*	*log meter-candles*	*minutes*	*minutes*
0.63	29.4	0.5	0.088	0.70	28.3	0.3	1.62	26.7	0.7
0.86	24.3	0.3	0.159	1.20	22.5	0.3	1.84	18.3	0.5
1.06	23.1	0.5	0.196	1.62	20.6	0.4	2.57	12.9	0.4
1.38	21.6	0.5	0.278	2.20	22.7	0.7			
1.67	20.7	0.3	0.370	2.57	28.2	0.9			
1.83	18.1	0.5	0.127	3.38	30.1	0.9			
1.92	16.8	0.7	0.152						
2.19	14.5	0.5	0.233						
2.37	13.7	1.0	0.286						
2.70	12.8	0.6	0.385						
3.09	13.1	0.3							
3.38	18.1	0.5							
3.88	23.0	0.7							

WHITE LIGHT AS STIMULUS

For finding the relation between intensity of light and reaction time, 10 determinations were made, using 10 separate etiolated plants, of the time of onset of bending at each of 13 intensities. The exposure was always 1 second. The data are given in Table I (coleoptile as a whole). The open circles in Fig. 2 represent these averages. Figure 2 shows that as the stimulating intensity increases the reaction time falls in two distinct and regular steps from the neighborhood of 30 minutes to about 13 minutes, then rises steeply. In the intensity range producing the rise in reaction time the shoot, after bending for about 9 minutes, very abruptly stops bending toward the light and begins bending away from it. Averages of four such responses at two intensities were recorded as follows: at log I = 3.38, $r. t.$ = 25.8; at log I = 3.88, $r. t.$ = 33.5. These are probably the negative responses observed by Arisz (1915) at intermediate intensities, the response sequence observed by him with increasing intensity being *positive—positive followed by negative—positive.*

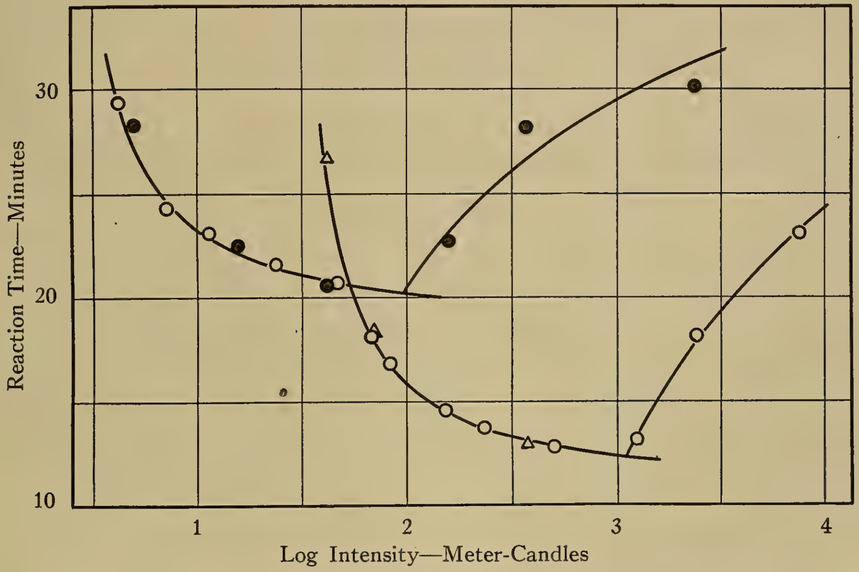


FIG. 2. Relation between log intensity of white light and reaction time. Each point is the average of 10 readings (Table I). Open circles, coleoptiles as a whole. Solid circles, bases shielded. Triangles, tips shielded. Descending curves are hyperbolas derived from the data. Ascending curves are theoretical.

The double relation between reaction time and intensity secured here is very likely an expression of the tip and base growth responses reported by Went. At 500 meter-candle-seconds, Went obtained a double growth response from the naked plant, one after 8 minutes and the other after 28 minutes. At this intensity (log I = 2.70) the average reaction time in my experiments is 12.8 minutes, only the earlier responses being recorded. Efforts to observe the expected second bending yielded such variable results that they were abandoned in favor of the procedure described in the next section.

As a purely empirical description of the data, the relation between reaction time and log intensity (up to the point of sharp increase in reaction time) may be represented by a rectangular hyperbola

$$(r - m)(\log I + C) = k,$$

where m and C are the asymptotes of reaction time and log I axes respectively, r is reaction time, and k a constant. The two descending curves in Fig. 2 are such hyperbolas; the equation for the upper data is

$$(r - 18)(\log I - 0.30) = 3.75,$$

and that for the lower is

$$(r - 10.2)(\log I - 1.41) = 3.35.$$

It is apparent that the data are precisely described by these curves. All measurements of reaction time against log I follow such a rectangular hyperbola, as will be made evident in the course of this paper.

Anatomical Separation by Shielding

If the double relation between reaction time and intensity shown in Fig. 2 represents the separate sensitivities of the base and tip of the plant, it should be possible to separate them by mechanically confining the illumination to one or the other of the two parts of the plant. Experiments showed this supposition to be correct. The base was shielded by placing a tube of tin-foil around all but 1.5 mm. of the tip, and the tip was shielded by dipping 3 mm. of it in a water suspension of lamp black until the dried coating was quite opaque. Otherwise the exposure and measurements were made as before.

Average reaction times for several intensities are presented in the second and third sections of Table I. The solid circles of Fig. 2 represent readings obtained by illumination of the tip only, and the triangles those resulting from stimulation of the base alone. It is apparent that the measurements made with partial illumination not only fall on the two curves calculated from data on naked plants, but yield information quite unobtainable except by a shielding procedure. Whereas with naked plants the upper data could not be extended beyond the point at which the lower curve begins, with plants whose bases are shielded this is possible. A free-hand curve drawn through the upper data for intensities greater than 100 meter-candle-seconds would indicate a reaction time at log $I = 2.70$ of about 29 minutes, while Went's reading at this intensity was about 28 minutes. On the lower curve, as with the upper data, shielding the tip made possible the reading at log $I = 1.62$ (26.7 minutes).

SEPARATION BY COLOR FILTERS

The data seem to provide clear evidence that there are two photoreceptor systems with separate loci in the tip and base of the coleoptile, and that these two systems function at different intensity levels. On the chance that they may also differ in regard to spectral sensibility, an attempt was made to separate them by the use of color filters. For this purpose Wratten filters were placed in the path of the light beam at a point near the plant. The experimental procedure was exactly as described for the study of naked plants stimulated by white light.

TABLE II

Reaction time to light from three different spectral regions. Whole coleoptile illuminated. Average of six readings.

Filter No.	Log relative intensity	Exposure time	Relative log $(I \times t)$	Average reaction time	Probable error
		seconds		*minutes*	*minutes*
76	1.42	1	1.42	25.7	0.4
	0.47	10	1.47	27.5	0.5
	0.69	10	1.69	18.8	0.5
	1.75	1	1.75	17.2	0.6
	1.42	10	2.42	13.3	0.5
	2.73	1	2.73	12.0	0.6
	1.75	10	2.75	12.7	0.8
	2.04	10	3.04	14.5	0.9
	2.73	10	3.73	20.5	0.9
75	0.84	1	0.84	30.5	0.8
	1.20	1	1.20	22.8	1.1
	1.90	1	1.90	20.8	0.8
	2.88	1	2.88	19.8	0.6
	2.38	10	3.38	14.0	0.8
	2.88	10	3.88	12.8	0.7
12	3.33	10	4.33	31.5	0.9
	3.76	10	4.76	22.7	0.8
	4.24	10	5.24	21.0	0.5
	4.60	10	5.60	17.0	0.8
	4.74	10	5.74	15.0	0.3
	4.74	30	6.22	12.3	0.4

Measurements made with the three filters are presented in Table II, and the plotted averages in Fig. 3. The abscissas of Fig. 3 are in relative energy units determined as previously described from measurements of the energy distribution of the lamp and the transmission of the filters.

The filters absorbed such large quantities of light that it was found necessary in the blue-green and minus-blue regions to increase the exposure time to 10 seconds, and in one case to 30 seconds, in order to obtain sufficient energy to complete the curves. This involves the assumption that the Bunsen-Roscoe relation holds for *Avena*. Justification for this assumption is supplied by Blaauw's (1909) results, which indicate that for a given bending response of this plant the product of intensity and time is a constant over enormous ranges of intensity and time. But in order to be quite sure that the procedure was sound, complete 1 and 10-second exposure curves were obtained for violet light (Filter No. 76). It will be seen from Fig. 3 that the 10-second readings (solid triangles) agree very well with those resulting from 1-second exposures (open triangles).

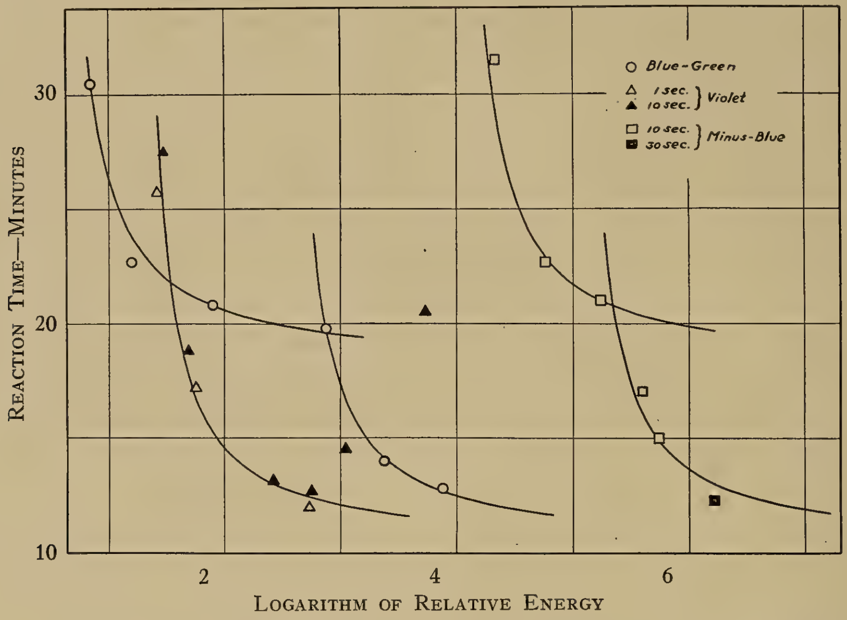


Fig. 3. Relation between reaction time and log relative energy for responses of *Avena* to three regions of the spectrum. Each point is the average of 6 measurements on naked coleoptiles (Table II). Circles, filter 75 (blue-green); squares, filter 12 (minus-blue); triangles, filter 76 (violet). Open squares and circles represent 10-second exposures, and the solid square a 30-second one. The open triangles represent 1 second and the solid triangles 10-second exposures: Note that they all fall on the same curve.

Figure 3 also reveals that no tip curve could be obtained when the violet filter was used, indicating that in the spectral region transmitted by this filter, base responses have lower thresholds than tip responses.

Such a filter thus affords a means of separating the two types of response without shielding. On the other hand, in the blue-green and minus-blue regions the tip is more sensitive than the base, as is the case when white light is the stimulus.

As before, all curves are drawn as rectangular hyperbolas, and it is apparent that they adequately described the data. The three base curves may be superimposed by shifting them along the axis of abscissas. In the same manner the tip curves may be superimposed. The displacements necessary in each case are measures of the relative effectiveness of the three kinds of light. Measurement of these displacements reveals that for base responses blue-green is 1,000 times and violet 18,000 times more effective than minus-blue light, and for tip responses blue-green is 3,000 times more effective than minus-blue. These differential sensitivities to color are sufficient to show a considerable difference between the slopes of the tip and base spectral sensibility curves included in these wave-lengths; the tip curve having the greater slope. The data also indicate a maximum sensibility in the violet or ultra-violet for base responses, and in the blue for tip responses.

MONOCHROMATIC LIGHT

Because of these findings it seemed profitable to measure the spectral sensitivities of the two processes in greater detail. This was done in the visible range only, using a monochromator as already described. To measure tip responses in those spectral regions to which the basal portion of the coleoptile is more sensitive than the tip, all but 1.5 mm. of the tip was shielded from the light. Energies were kept below the point at which an increase in reaction time occurs.

Table III contains measurements obtained at each wave-length. Curves *a*, *b*, *c*, *d*, and *i* of Fig. 4 are pure tip curves obtained by shielding the coleoptile bases; curves *j*, *k*, *l*, *p*, *q*, and *r* are pure base curves (naked coleoptiles) secured in spectral regions where tip sensibility is lower than base sensibility. Curves *e* and *m*, *f* and *m*, *g* and *n*, and *h* and *o* are separately plotted double curves in spectral regions of lower base sensitivity. Note that curve *m* passes through data from two spectral bands. It was assumed that the single points of curves *q* and *r*, and the two points of curve *h* pass through hyperbolas identical with those of the remainder of the data. As before, all curves are rectangular hyperbolas. The equation for the upper data is

$$(r - 18)(\log I + C) = 3.75,$$

and that for the lower

$$(r - 10.2)(\log I + C) = 3.35,$$

where C depends upon the position of the curve on the log energy axis.

TABLE III

Responses to 12 wave-lengths. Average of 6 determinations. Exposure time 10 seconds.

Coleoptiles as a whole				
Wave-length	Log relative energy	Average reaction time	Probable error	Curve
Å		*minutes*	*minutes*	
4092	0.88	30.0	0.6	*j*
	1.02	20.3	0.3	
	1.23	16.2	0.3	
	1.46	14.7	0.8	
4460	2.28	24.8	1.0	*k*
	2.48	18.2	0.6	
	2.67	15.2	0.5	
	2.87	14.3	1.4	
4698	2.34	27.7	0.7	*l*
	2.44	20.8	0.6	
	2.75	16.0	0.5	
	3.16	13.2	0.3	
4735	2.76	29.2	0.3	*e*
	2.82	26.8	0.6	
	2.95	23.8	0.9	
	3.13	21.8	0.6	
	3.32	20.0	0.4	*m*
	3.41	18.2	0.6	
	3.51	16.2	0.6	
	3.89	13.7	0.5	
4800	2.58	24.8	0.7	*f*
	2.78	22.0	0.1	
	2.97	21.0	0.5	
	3.16	20.5	0.5	
	3.36	18.8	0.7	*m*
	3.55	15.7	0.4	
	3.74	14.2	0.3	
	3.93	13.2	0.2	
4850	2.58	31.3	0.6	*g*
	2.78	24.8	0.2	
	3.17	20.3	0.6	
	3.37	19.8	0.5	
	3.55	18.8	0.6	*n*
	3.75	15.5	0.5	
	3.94	14.3	0.4	
	4.33	12.8	0.3	

Coleoptiles as a whole (Cont'd)				
Wave-length	Log relative energy	Average reaction time	Probable error	Curve
Å		*minutes*	*minutes*	
4901	3.01	26.2	0.1	*h*
	3.07	24.8	0.3	
	3.21	23.5	0.8	
	3.40	21.2	0.3	
	3.79	19.5	0.4	
	3.98	18.0	0.4	*o*
	4.18	15.8	0.4	
	4.37	14.7	0.3	
	4.57	13.2	0.3	
5002	4.00	24.5	0.5	*p*
	4.20	18.3	0.4	
	4.40	15.3	0.4	
	4.90	13.8	0.4	
5155	5.56	24.8	0.5	*q*
5418	5.74	24.5	0.9	*r*
Bases of coleoptiles shielded				
4092	3.41	25.0	0.4	*a*
	3.50	23.5	0.3	
4302	3.33	30.0	0.4	*b*
	3.62	23.0	0.4	
	3.91	21.0	0.4	
	4.30	20.0	0.4	
4612	3.96	26.5	0.3	*c*
	4.15	22.7	0.3	
	4.34	21.2	0.2	
	4.52	19.7	0.5	
4698	3.06	26.7	0.3	*d*
	3.16	25.0	0.3	
	3.36	22.7	0.3	
	3.76	19.8	0.6	
5002	4.60	30.8	0.9	*i*
	4.70	26.0	0.4	
	4.80	23.5	0.9	
	5.21	20.8	0.4	

The relative energy required at a given wave-length in order to produce a specific bending effect is given by the distance of its curve from the curve at the extreme left of the figure which represents responses to the most effective light. The relative energies so obtained are given in Table IV and are illustrated graphically by Fig. 5 (large

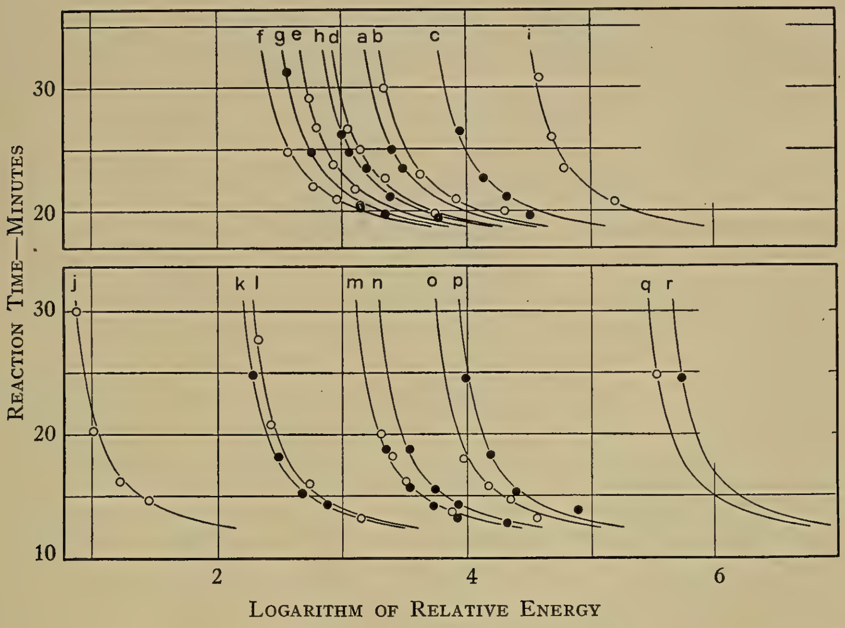


Fig. 4. Relation between reaction time and log relative energy for responses of *Avena* to narrow spectral bands. Each point is the average of single readings on 6 separate plants (Table III). Alternate open and solid circles are used to avoid confusion. The hyperbolas have the same constants as those describing the data from white and filtered light, but the values of the vertical asymptotes vary with their positions on the axis of abscissas.

Upper graph Tip		Lower graph Base	
Curve	λ	*Curve*	λ
a	4092	j	4092
b	4302	k	4460
c	4612	l	4698
d	4698	m	4735 4800
e	4735	n	4850
f	4800	o	4901
g	4850	p	5002
h	4901	q	5155
i	5002	r	5418

open and solid circles). For obtaining the vertical distance between these two curves, the energy required for a constant response by the tip and base for the same wave-length must be compared. Clearly the only part of the tip and base curves of Fig. 4 which may be regarded as comparable responses are the thresholds, i.e., the values of the asymptotes to the axis of ordinates. These were computed and the log *I* difference between them for each wave-length used to fix the relative position on the axis of ordinates of the two curves in Fig. 5. The ordinates in Fig. 5, when read from tip to bottom, give log relative energies. If read from bottom to top, they give log reciprocals of relative energies and therefore represent the relative effectiveness of the different parts of the spectrum.

TABLE IV

Relation of energy to wave-length, derived from the data of Fig. 4 by measuring the amount of displacement on the axis of abscissas required to superimpose each series of hyperbolas

Tip series			Base series		
Curve	Wave-length	Log relative energy	Curve	Wave-length	Log relative energy
	Å			Å	
a	4092	2.92	*j*	4092	0.71
b	4302	3.02	*k*	4465	2.04
c	4612	3.50	*l*	4698	2.14
d	4698	2.75	*m*	4735	2.96
e	4735	2.39	*m*	4800	2.96
f	4800	2.10	*n*	4850	3.14
g	4850	2.26	*o*	4901	3.60
h	4901	2.58	*p*	5002	3.80
i	5002	4.25	*q*	5155	5.32
			r	5418	5.51

The tip curve has a distinct maximum at λ4800, and a broad secondary one at the violet end of the spectrum. The base curve exhibits no maximum in the visible range. The high value at λ4092 would seem to point to a maximum in the ultra-violet. These results confirm the general conclusions drawn from the data obtained by the use of color filters.

Determinations of the relative effectiveness of different spectral regions on phototropic responses of *Avena* have been made by Blaauw (1909), Koningsberger (1922), Sonne (1929), Bergann (1930), and Johnston (1934). The quantity they measured was not, as in my experiments, the energy required for a constant reaction time in different parts of the spectrum, but rather the energy necessary for a specific amount of bending, or else the ratio of energies for no response to antagonized lights of different wave-lengths. Although Sonne stimulated only the first 10 mm. of the shoot, which was the average length of my plants (and therefore not the "tip" as here defined), in no case were the precautions I have described used to separate tip and base responses. It is therefore possible that tip responses were observed by these workers only in spectral regions to which the tip is more sensitive than the base.

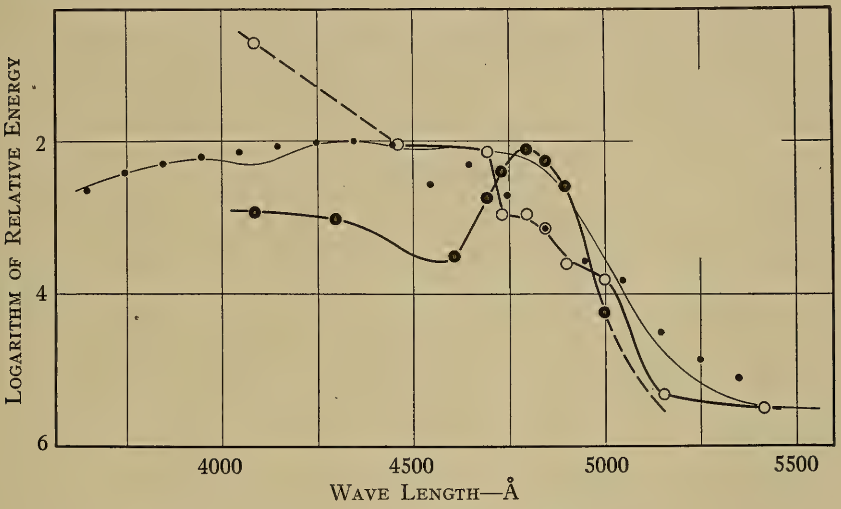


Fig. 5. Relation between wave-length and log relative energy of stimulus for tip and base responses (Table IV). Large open circles are derived from measurements of base responses and large solid circles from those of tip responses as described in text. The thin line represents the average measurements of Blaauw, Koningsberger, Sonne, and Johnston and is roughly fitted to the high portions of the base and tip data. The small solid circles are the data of Bergann.

The data of Blaauw, Koningsberger, Sonne, and Johnston, corrected when necessary as suggested by Bachmann and Bergann (1930), agree so closely when plotted logarithmically, that it seemed legitimate to average them for convenient comparison with my data. Except at λ4092, these averages (Fig. 5, thin line), as anticipated, fall on a curve roughly corresponding to the envelope of the tip and base curves. On the other hand, Bergann's data (small solid circles) approximate the base curve only; hence, Bergann's technique or else his criterion of a bending response must have excluded tip responses.

The absorption spectrum of a photochemical system is closely correlated with its chemical behavior. To what extent the spectral sensibility of a tropistic reaction may approximate the absorption spectrum of the photic mechanism involved depends upon a variety of factors, e.g., the extent of masking by extraneous pigments, the solution state of the photoreactants, the optical conditions at the receptor. Except that pigment density in the coleoptile of *Avena* appears to increase slightly from the apex downward, principally after illumination, we have no reason to think that these conditions are not almost identical at the tip and base receptors. Therefore the differences between the shapes of the two curves in Fig. 5 probably represent real differences in the absorption spectra of two photochemical systems.

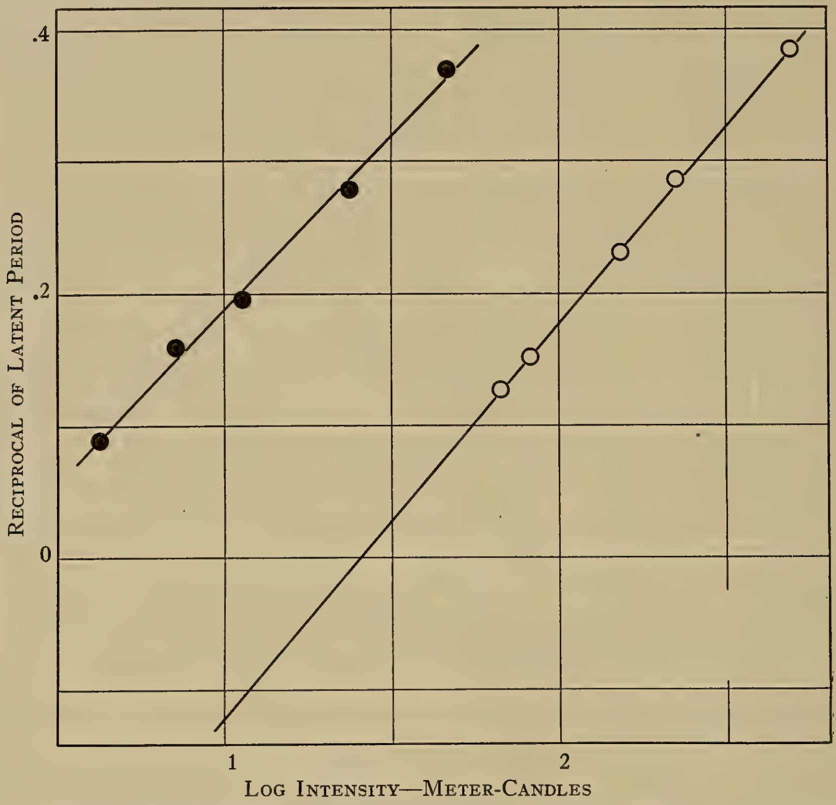


FIG. 6. Relation between $1/(r - m)$ [velocity of latent period reaction] and log intensity of white light for tip and base responses of naked plants. Solid circles, tip data; open circles, base data. The values of $1/(r - m)$ are found in Table I. The equations are given in the text.

THEORETICAL

The simple reciprocal relation found in all my measurements between log I and reaction time suggests certain theoretical possibilities. The data here presented furnish information about the time elapsing before bending begins. The shorter the reaction time the faster must be the preliminary process leading up to bending. Hence, the reciprocal of the reaction time is a measure of the speed of this preliminary process. The reaction time apparently has a limit m below which it never goes. A plot of $1/(r - m)$ against log I yields a straight line (Table I and Fig. 6), indicating direct proportionality between log I and the speed of the process occurring in the interval $(r - m)$[latent period]. This is a corollary of the hyperbolic relation between log I and $(r - m)$. The equations are

$$1/(r - 18) = 0.267 \log I - 0.081$$

for the tip, and

$$1/(r - 10.2) = 0.299 \log I - 0.422$$

for the base. The hyperbolas of Figs. 2, 3, and 4 may be calculated from these equations, the constants being reciprocals of those appearing in the purely mathematical treatment of the data.

An identical relation between log I and reciprocal of latent period has been found for the photic responses of *Mya* (Hecht, 1920) and *Pholas* (Hecht, 1927), and a similar function appears in the photogrowth and phototropic reactions of *Phycomyces* (Castle, 1930). In animals the constant period m is occupied by the events of the reflex arc and is negligibly small. In plants m is very large and is probably occupied by extra-receptor preparations for bending as well as by the early unobserved bending.

We may suppose that there are photo-labile substances in the coleoptile (the inclosed first leaf being insensitive, Rothert, 1896; Paál, 1919) that are decomposed by light during the very brief exposure period. Their decomposition products determine the speed of a secondary non-photic reaction whose products start a chain of events leading up to growth inhibition when the illumination is general and to bending when the illumination is one-sided. If we assume that the velocity of the second reaction is directly proportional to the concentration of photoproducts, then the fact that $1/(r - m)$ plotted against log I yields a straight line shows the concentration of photoproducts to be a logarithmic function of intensity, a relationship common in reversible photochemical systems possessing stationary states. Apparently there are two such sensitive systems present in *Avena*.[3]

---

[3] Strict adherence to this view of the photomechanism would require that the exposure period as well as m be subtracted from the reaction time, but since my calculations are based on experiments with 1-second exposures, the maximum possible error introduced by this omission is less than 0.15 per cent.

The fact that the growth of seedlings is slower in light than in darkness has been made the basis of a theory of phototropism first suggested by de Candolle (1832) and later elaborated by Blaauw (1914, 1915, 1919). Stated in general terms, the explanation for the bending of a shoot given by Blaauw's theory is that the effect of light is to inhibit growth through the mediation of a photochemical reaction; hence if more light reaches one side than the other its growth rate is diminished to a greater extent and the result is a bending toward this side.

Castle (1930, 1933*a*, *b*) has shown that this explanation can be applied without serious modification to the bending responses of *Phycomyces*. In this case the plant grows faster in light than in darkness, but unilateral illumination nevertheless causes positive bending because the optical characteristics of the plant are such that light is focused on the far side. That vertical growth and bending in *Phycomyces* are fundamentally the same phenomenon is demonstrated by the fact that reaction times for both responses are parallel and almost equal over a wide range of light exposures.

The conditions in seedlings are more complicated than originally supposed by Blaauw. It has been shown by F. W. Went (1926, 1928) and confirmed by others (van Overbeek, 1933; F. A. F. C. Went, 1935) that cells in the coleoptile tip produce a growth-promoting substance, auxin, which moves downward into the coleoptile base. The coleoptile may be "decapitated" and the tip replaced by a block of agar impregnated with auxin which in many respects behaves toward the remaining coleoptile stump as though it were the original tip. The stream of down-moving auxin is deflected to the unlighted side of the stump when the latter is unilaterally illuminated. This causes the plant to bend toward the light. In addition, van Overbeek (1933) has shown for *Raphanus* that the sensitivity of the stump cells to the growth-promoting action of auxin is reduced by light. This latter effect very likely causes the base growth responses recorded by Went. The base bending responses observed in the present experiments could thus be caused by a decrease in sensitivity to auxin on the lighted side supplemented by a deflection of auxin to the unlighted side.

The effects of illumination on the tip are somewhat different. A number of observations had suggested and F. W. Went (1926) demonstrated on *Avena* that as the intensity of light falling on the excised tip is increased up to 1,000 meter-candle-seconds the amount of auxin coming out of the tip is diminished.[4] Further increases in intensity cause an increase in auxin. (For other examples of an increase in auxin on illumination, see Thimann and Dolk, 1933).

---

[4] Unless one is prepared to accept the older view that products of the photoreceptor process formed in the tip move down to the base and there inhibit growth, a

Thus with the naked plant, at low intensities one would expect positive responses to unilateral illumination due to a decrease in the amount of auxin coming down from the tip on the illuminated side (tip responses). At higher intensities, but not exceeding 1,000 meter-candle-seconds, one would expect base responses involving auxin deflection to the distal side and decrease of sensitivity to the action of auxin on the proximal side. (These occur earlier than the tip responses and in reaction time studies are the only ones recorded at these intensities.) At intensities just above 1,000 meter-candle-seconds the plant should first give positive base responses (for the reasons just given) and then after a few minutes, negative responses due to the accumulation of additional auxin on the proximal side of the tip. If the tip is shielded, the negative responses should not occur (Arisz actually observed this). At still higher intensities positive base responses not followed by negative ones might be expected to appear provided the accumulation of auxin in the tip reaches its maximum at these intensities and the inhibitory and deflecting effects of illumination of the base are again able to dominate.

Arisz' results and the variations in reaction time and direction of response with increasing intensity here recorded are entirely consistent with these deductions. This is perhaps less obviously true of the rise in reaction time at intensities above 1,000 meter-candle-seconds than of the regular decreases and the negative responses. That reaction times should rise as they do at these intensities may be readily seen by assuming with Blaauw, as indeed we must in view of the present findings regarding the significance of the reaction time, that the products of a photochemical reaction control the speed of a secondary process whose products set in motion the bending mechanism. It then follows that more time will be required (i.e., longer reaction times) for accumulation of the necessary concentration of secondary products to deflect the greater quantity of auxin present at these intensities.[5]

---

general reduction in the outflow of auxin from the tip such as Went observed must be supposed to occur in order to explain the inhibition of vertical growth rate on general illumination of the tip alone. However, the existing evidence on this point does not entirely rule out the older conception, at least as a concomitant process.

[5] In this outline the possible influence of the less intense light which reaches the distal side of the coleoptile has been neglected. Van Dillewijn (1925) has estimated this to be about 1/30 of the proximal intensity. Hence some effect should be noted at about 30 times the threshold intensity. It happens that this is almost precisely the intensity at which the rise in reaction time begins to appear. This may be no more than a misleading coincidence because, while a decrease in total bending is to be expected and was indeed observed (Arisz, 1915), on the other hand a lengthening

A fact which should repay further study is that the difference in value of the constant m appearing in the tip and base reaction time equations ($18 - 10.2 = 7.8$ minutes) is very nearly the average difference in reaction time (9 minutes) between positive and negative responses. These differences should be equal if, as we suppose, they both represent the time necessary for a change in the quantity of auxin flowing from the tip to be registered as a bending of the basal zone of the coleoptile.

Finally, the fact that the tip and base bending mechanisms are probably not the same is consistent with the differences here found between the absorption spectra of the photosensitive substances located in these two zones.

The fact here established that the photic mechanism underlying the response of *Avena* to light may be given a simple chemical interpretation not unlike those applying to several other organisms is neither surprising nor of special significance. Of more immediate importance to future studies of this plant is the observation that it possesses two anatomically separable photic systems functioning at different intensity levels and having different absorption spectra. The analogy of this to the rod and cone mechanism of the vertebrate retina is obvious. The rods and cones are anatomically distinct, function at different intensity levels, and show a certain difference in their spectral visibility curves. Until these facts about the eye were established, the data on vision could not be properly evaluated. Since *Avena* is so widely used in studies of the light reactions of plants, it is important to know that it has a similarly dual photoreceptor mechanism.

SUMMARY

The reaction time for positive phototropic responses of *Avena sativa* for short exposures to white light decreases with increasing intensity up to about 1,000 meter-candles, and then increases. The response curve shows two parts, indicating a double photoreceptor process. The two response curves are rectangular hyperbolas, from which it is deduced that the speed of the initial reaction time process is a logarithmic function of intensity.

---

of the reaction time from this circumstance involves the rather improbable condition that a subliminal concentration of distal photoproducts is able to produce an effect resembling a reduction in the concentration of proximal photoproducts. It is nevertheless possible to describe the reaction time data for these intensities in terms of these ideas by assuming that the final latent period velocity $[1/(r - m)]$ at a given illumination is proportional directly to the proximal light effect and inversely to the distal effect, and that the coleoptile transmission is 1/34. The two ascending curves of Fig. 2 were computed in this manner.

When only the first 1.5 mm. of coleoptile tip is stimulated, only one response curve is obtained. When all but the upper 3 mm. of coleoptile is exposed, the other single curve is obtained. Hence the two photoreceptor systems have separate loci.

The relation between reaction time and relative energy found when homogeneous lights of various wave-lengths fall on naked and partly shielded coleoptiles yields two distinctly different spectral sensibility functions, a tip and a base response. While Bergann seems to have observed only base responses, the single functions reported by four other investigators appear to represent tip responses in spectral regions of greater tip sensibility and base responses in regions of greater base sensibility.

The bearing of these findings on the theory of phototropism is discussed.

It is with pleasure that the writer expresses his obligation and gratitude to Professor Selig Hecht for guidance and encouragement throughout this investigation.

Thanks are also due Professor Sam F. Trelease of the Department of Botany for many helpful suggestions regarding the plant literature, and Mr. Simon Shlaer of the Laboratory of Biophysics for advice and collaboration in the construction and calibration of the apparatus.

BIBLIOGRAPHY

ARISZ, W. H., 1915. Untersuchungen über den Phototropismus. *Rec. trav. bot. néerl.*, **12**: 44.

BACHMANN, F., AND F. BERGANN, 1930. Über die Wertigkeit von Strahlen verschiedener Wellenlänge für die phototropische Reizung von Avena sativa. *Planta, Arch. wiss. Bot.*, **10**: 744.

BERGANN, F., 1930. Untersuchungen über lichtwachstum, Lichtkrümmung und Lichtabfall bei Avena sativa mit Hilfe monochromatischen Lichtes. *Planta, Arch. wiss. Bot.*, **10**: 666.

BLAAUW, A. H., 1909. Die Perzeption des Lichtes. *Rec. trav. bot. néerl.*, **5**: 209.

BLAAUW, A. H., 1914. Licht und Wachstum, I. *Zeitschr. Bot.*, **6**: 641.

BLAAUW, A. H., 1915. Licht und Wachstum, II. *Zeitschr. Bot.*, **7**: 465.

BLAAUW, A. H., 1919. Licht und Wachstum, III. Die Erklärung des Phototropismus. *Mededeilingen Landbauwhoogesch., Wageningen.*, **15**: 89.

DE CANDOLLE, A. P., 1832. Physiologie végétale. Paris, Bechet (jeune), Tome **3**: 1083.

CASTLE, E. S., 1930. The light-sensitive system as the basis of the photic responses of *Phycomyces*. *Proc. Nat. Acad. Sci.*, **16**: 1.

CASTLE, E. S., 1933*a*. The refractive indices of whole cells. *Jour. Gen. Physiol.*, **17**: 41.

CASTLE, E. S., 1933*b*. The physical basis of the positive phototropism of *Phycomyces*. *Jour. Gen. Physiol.*, **17**: 49.

DARWIN, C., 1880. The Power of Movement in Plants. London, Murray.

VAN DILLEWIJN, C., 1925. The connection between lightgrowthresponse and phototropical curvature of seedlings of Avena sativa. *Kon. Akad. Wetensch. Amst.*, **28**: 775.

FORSYTHE, W. E., 1920. 1919 Report of Standards Committee on Pyrometry. *Jour. Opt. Soc. America*, **4**: 305.

HAIG, C., 1934*a*. The effect of intensity and wave-length on the response of Avena to light. *Proc. Nat. Acad. Sc.*, **20**: 296.

HAIG, C., 1934*b*. The spectral sensibility of Avena. *Proc. Nat. Acad. Sci.*, **20**: 476.

HECHT, S., 1920. Intensity and the process of photoreception. *Jour. Gen. Physiol.*, **2**: 337.

HECHT, S., 1927. The relation of time, intensity, and wave-length in the photosensory system of Pholas. *Jour. Gen. Physiol.*, **11**: 657.

JOHNSTON, E. S., 1934. Phototropic sensitivity in relation to wave-length. *Smithsonian Misc. Collections*, No. 11 (Publ. 3285) **92**: 1.

KONINGSBERGER, V. J., 1922. Tropismus und Wachstum. *Rec. trav. bot. néerl.*, **19**: 1.

VAN OVERBEEK, J., 1933. Wuchsstoff, Lichtwachstumreaktion, und Phototropismus bei Raphanus. *Rec. trav. bot. néerl.*, **30**: 537.

PAÁL, A., 1919. Über phototropische Reizleitung. *Jahrb. wiss. Bot.*, **58**: 406.

ROTHERT, W., 1896. Ueber Heliotropismus. *Beitr. z. Biol. d. Pflanzen*, **7**: 1.

SIERP, H., AND A. SEYBOLD, 1926. Untersuchungen über die Lichtempfindlichkeit der Spitze und des Stumpfes in der Koleoptile von Avena sativa. *Jahrb. wiss. Bot.*, **65**: 592.

SONNE, C., 1929. Weitere Mitteilungen über die Abhängigkeit der lichtbiologischen Reaktionen von der Wellenlänge des Lichts. *Strahlentherapie*, **31**: 778.

THIMANN, K. V., AND H. E. DOLK, 1933. Conditions governing the production of the plant growth hormone by Rhizopus cultures. *Biol. Zentr.*, **53**: 49.

WENT, F. A. F. C., 1924. Communication on Miss A. Bakker's "Investigations regarding the existence of separate zones of perception and reaction in the seedlings of Paniceæ." *Proc. Sec. Sci., Kon. Akad. Wetensch. Amst.*, **27**: 501.

WENT, F. A. F. C., 1935. The investigations on growth and tropisms carried on in the Botanical Laboratory of the University of Utrecht during the last decade. *Biol. Rev.*, **10**: 187.

WENT, F. W., 1925. Concerning the difference in sensibility of the tip and base of Avena to light. *Proc. Sec. Sci., Kon. Akad. Wetensch. Amst.*, **29**: 185.

WENT, F. W., 1926. On growth-accelerating substances in the coleoptile of Avena sativa. *Proc. Sec. Sci., Kon. Akad. Wetensch. Amst.*, **30**: 10.

WENT, F. W., 1928. Wuchstoff und Wachstum. *Rec. trav. bot. néerl.*, **25**: 1.

PROGRAM AND ABSTRACTS OF SCIENTIFIC PAPERS PRESENTED AT THE MARINE BIOLOGICAL LABORATORY

July 2, 1935

EFFECTS OF ALCOHOL ON NERVES. Carl Caskey Speidel.

Practically any degree of neuritis may be induced in living frog tadpoles if these are immersed in dilute solutions of alcohol for suitable periods of time. Direct observations on living alcoholized tadpoles reveal that irritated myelinated fibers exhibit vacuolation, fibrillation, swelling, globule formation, and in extreme cases complete degeneration of some myelin segments. The node of Ranvier, however, resists swelling. Neurofibril-like structures become visible during the early stages of neuritis, as well as during the process of reduction and recovery of swollen fibers following irritation. Myelin globules cut off during irritation are readily re-incorporated into the myelin sheath during recovery.

Prolonged irritation causes complete degenerative metamorphosis of the more distal, but not the more proximal, myelin segments of a fiber. Mild daily intoxication of brief duration, even though continued for many weeks, causes little damage to nerves. The slight irritative changes that appear each day during treatments are quickly repaired. Under these conditions nerve growth continues, including myelin ensheathment of fibers and the origin and extension of new nerve branches.

The growing tips of regenerating fibers respond to alcohol treatment by marked retraction. Quick recovery with new growth follows replacement of the animal in pond water.

Irritated cutaneous nerve endings exhibit swelling, retraction, and occasionally autotomy. Essentially, the processes of retraction and recovery are like those of regenerating nerve tips, though they are less conspicuous.

Unmyelinated fibers ensheathed with neurilemma, and sheath cells of Schwann, except for the presence of an occasional vacuole, exhibit little change during irritation. Sheath cells may divide mitotically without injury in animals under treatment with alcohol of sufficient strength to cause marked irritation of myelinated fibers.

Ciné-photomicrographs of both the normal and fast motion types have been obtained which record the principal changes in nerve fibers during alcoholic neuritis and recovery.

SUCCESSIVE INJURY AND RECOVERY OF STRIATED MUSCLE. Carl Caskey Speidel.

With suitable electrical stimulation any degree of injury may be induced in a fiber of striated muscle in a living frog tadpole. A single fiber was successively injured several times and the process of repair observed after each injury. Motion pictures, taken daily, record in minute detail the various structural changes in the fiber during its entire history of injury and recovery, as follows:

April 3: moderate injury with conspicuous "retraction cap" and loss of striæ at one end, followed by liquefaction of retraction cap, elongation, and development of new cross striæ.

April 5: severe injury with retraction, fibrillation, swelling, loss of all cross striæ, and ultimate phagocytosis of everything except a few muscle nuclei, followed by stages in regeneration including myoblast mitosis, myotube formation without cross striæ, origin and growth of myofibrils, cross striæ, and of the fiber as a whole.

April 17: slight injury with some fibrillation, swelling, and loss of a few cross striæ at one end, followed by rapid repair.

April 18: moderate injury with conspicuous retraction cap, loss of some cross striæ, fibrillation, differential slipping of groups of myofibrils with loss of perfect alignment of cross striæ, followed by recovery during the next two days with restoration of normal conditions.

April 20: injury during treatment of the tadpole with hypertonic solution of sodium chloride.

An abnormal type of muscle contraction resembling that of arthropods is caused by suitable treatment with solutions of sodium chloride or calcium chloride. This type of contraction exhibits two striking features: (1) the zone of contraction is sharply localized, covering only about a dozen striation units; (2) the node of contraction advances relatively slowly along the fiber. It may proceed in either direction.

OVULATION IN THE FROG. Roberts Rugh.

July 9

THE LIBERATION OF ENERGY DURING THE SIMPLE TWITCH OF SKELETAL MUSCLE. Dugald E. S. Brown.

THE EFFECT OF ORGANIC IONS ON THE NERVE MEMBRANE POTENTIAL. W. Willbrandt.

RESPIRATORY POISONS AND THE FREQUENCY OF THE EMBRYONIC FISH HEART. Kenneth C. Fisher and Laurence Irving.

RESPIRATORY METABOLISM DURING CARDIAC INHIBITION. W. E. Garrey.

THE RESPIRATORY METABOLISM OF THE SEAL. Laurence Irving.

July 16

SOME SURFACE PHENOMENA IN CENTRIFUGED SEA URCHIN EGGS. Ethel Browne Harvey.

THE EFFECTS OF CENTRIFUGING THE EGGS OF NUDIBRANCHS. Donald P. Costello.

The fertilized eggs of a number of Pacific Coast nudibranchs were centrifuged with very high centrifugal forces during maturation and early cleavage. The polar bodies were induced to form at the hyaline zone by centrifuging before the maturation divisions. In some cases the early cleavage planes passed through the induced position of the maturation body rather than through the pole of the egg. Hyaline fragments of these eggs occasionally cleaved with the normal spiral cleavage pattern.

THE PERMEABILITY OF THE NUCLEUS OF AMEBA TO DYESTUFFS. L. Monné.

CORTICAL CHANGES IN CELL DIVISION. Robert Chambers.

July 30

QUANTITATIVE STUDIES ON THE RATE OF REGENERATION OF TUBULARIA. L. G. Barth.

EDITOR'S NOTE: The concentrations of potassium bi-chromate given in the abstract of the paper by Oscar W. Richards (p. 372 of the October issue) should read μg./ml. instead of mg./ml.

SEX-MODIFICATION IN THE CHICK EMBRYO RESULTING FROM INJECTIONS OF MALE AND FEMALE SEX-HORMONES. B. H. Willier, R. F. Gallagher, and F. C. Koch.

PHYSIOLOGY OF OVULATION AND EJACULATION IN THE OYSTER. Paul S. Galtsoff.

August 6

THE PERMEABILITY OF THE ERYTHROCYTE TO HEAVY WATER. A. K. Parpart.

KILLING ORGANISMS WITH CHROMIUM AS FROM INCOMPLETELY WASHED BICHROMATE-SULPHURIC CLEANED GLASSWARE. Oscar W. Richards.

Concentrations of from 0.1 to 1.0 mg./ml. of bichromate may occur when small amounts of water are used in dishes which have been cleaned with potassium bichromate sulfuric acid cleaning fluid and washed with seven changes of tap water and three of distilled water. Laug has further demonstrated the difficulty of removing these remaining traces of bichromate. The above amounts of potassium bichromate are shown to be sufficiently toxic to invalidate experiments made with yeast, *Oscillatoria*, two species of *Spirogyra*, and developing *Amblystoma* eggs. A desmid and a diatom were less affected by the bichromate but the former (*Raphidium*) was injured by 1.0 mg./ml. of bichromate. Only 50 per cent of the *Amblystoma* hatched as normal tadpoles when the bichromate concentration was only 0.0001 mg./ml. Unless it is known that the washing of glassware cleaned in this mixture is adequate, or that the organisms used are not poisoned by such traces as may remain, it is recommended that less toxic cleaning solutions be used. The cleaning of micro-respiration apparatus should receive special attention. Other cleaning materials that wash off more easily are Sapolio, 10 per cent nitric acid or 1 to 5 per cent trisodium phosphate.

THE EFFECT OF OXYGEN CONSUMPTION ON ALCOHOLIC FERMENTATION IN YEAST EXTRACTS. C. V. Smythe.

THE SCOPE OF THE CHEMICAL METHOD OF ESTIMATING HEMOGLOBO-CARBAMATE. J. K. W. Ferguson.

In hemoglobin solutions to which alkali and $BaCl_2$ have been added, hemoglobocarbamate slowly dissociates; the CO_2 changing to $\overline{CO_3}$. When this process is complete the addition of Na_2CO_3 to provide a more bulky precipitate allows the complete centrifuging down of the $\overline{CO_3}$ as $BaCO_3$. It may then be concluded that the chemical method of Ferguson and Roughton (*J. Physiol.*, 83, 1934) is applicable even when the total CO_2 content of the original solution is low. The rate of dissociation of $HbCO_3$ at about pH 12 has been studied at different temperatures. It seems evident that considerable loss of $Hb\text{–}CO_2$ must occur during an analysis. Previously published results may be as much as 25 p.c. low.

The method has been applied to solutions of human hemoglobin. The conclusions drawn from experiments on beef hemoglobin have been confirmed.

DACRYORRHETIN: DEMONSTRATION OF ITS ACTION BY A KODACHROME FILM. Shiro Tashiro and Helen Stix.

A substance or a fraction was isolated from dogfish muscle which has the property of making an animal weep when injected into the intraperitoneal cavity. To designate this property we have named this compound *dacryorrhetin* (G. *dacryo*, tear; *rhoia*, flow), a term derived for me from Greek by Mr. R. A. Brower, of Harvard. A similar compound has also been isolated from beef steak and steer's blood; Miss Stix has prepared it from beef's heart. The motion picture which Miss Stix and I prepared for the purpose of demonstrating this action will show in the natural colors the following points: the weeping of "milky tears" by guinea pigs and rabbits and of light "milky tears" by mice; the shedding of "bloody tears" by rats; salivation by chicks and a cat; a temporary paralysis in guinea pigs and chicks. The other actions such as causing copious flow of ordinary saline tears in cats and dogs, and producing gastric ulcers in guinea pigs are not included in this film.

August 13

A Consideration of Biological Factors Influencing the Radiosensitivity of Cells. Paul S. Henshaw.

It has often been observed that cells and organisms which are more active in growth and mitosis are more vulnerable to short wave-length radiations such as X-rays. This has given rise to the generalization that the radiosensitivity of cells follows hand-in-hand with metabolic rate and biological activity in general. Further, the belief exists that organisms are more vulnerable to radiation because they are more active biologically.

Experiments have been carried out to investigate these relationships. Growth activity in a certain organism, *Triticum vulgare* Villars (wheat), was caused to vary over wide ranges by various means and the susceptibility to radiation determined during the intervals when growth rate differed widely. This was done in an attempt to determine whether the radiosensitivity varied in the same way and at the same time. The results obtained showed that it followed growth activity closely in some cases, less in other cases, and little or none in others. Further experiments showed that changes in radiosensitivity take place quite independently of biological behavior such as mitotic activity, respiratory rate, and water uptake. From this it is apparent that while radiosensitivity and certain forms of biological activity may often go hand-in-hand, these factors are not inseparably connected.

Since radiation, as such, does not act on activity but upon matter—the protoplasmic constituents of which living objects are composed, it is clear that the radiosensitivity is associated with the more inherent properties of cells, first their constituents and how they are organized and then how such organization varies from time to time. Correlating radiosensitivity with any biological activity is, therefore, the first of a two-phase problem and the experiments described have dealt with the first phase only. The second phase is a matter of showing that the particular biological activity in question is an expression of the protoplasmic constituents which are affected by the radiation.

Since radiation acts only on matter and not activity, it is clear that organisms cannot be more vulnerable to this agent simply because they are more active biologically in some way or other.

The Significance of Mid-Bodies. Henry J. Fry.

The Intergeneric Homology of a Euchromosome in Several Closely Related Acridinæ. J. G. Carlson.

Phylogenetic Significance of Some Structural Conditions in the Orthoptera. C. E. McClung.

August 20

ON THE NUTRITIONAL REQUIREMENTS OF MOSQUITO LARVÆ. William Trager.

THE EXPERIMENTAL PRODUCTION OF TUMORS IN RATS THROUGH CELLULAR MALNUTRITION. J. E. Davis.

DARK ADAPTATION IN THE INSECT DINEUTES ASSIMILIS. Leonard B. Clark.

The course of dark adaptation was followed in the aquatic beetle, *Dineutes assimilis*, by finding the threshold intensity to which specimens would react after various measured periods in the dark. Observations were made after light adaptation to intensities of 6.5, 91.6 and 6100 foot candles.

The results are described by an equation similar to that of a bimolecular isotherm. On analysis the average values of the velocity constant were 1.324, 1.477, and 1.282 for the three series of data. The average for all readings was 1.326.

PROTEIN LIPID BINDING IN PROTOPLASM. L. V. Heilbrunn.

When an *Arbacia* egg is centrifuged, the light fatty constituents of the protoplasm are massed at the centripetal pole to form the so-called gray cap or oil cap. This cap is normally very small. If, however, eggs are exposed to mixtures of sea water and isotonic solutions of ammonium salts and are then centrifuged at varying intervals, the oil cap increases greatly in volume, until after 8 or 10 hours it occupies a considerable fraction of the egg. Various salts of ammonium all produce this effect. Thus chloride, sulphate, acetate, and oxalate were successfully used. The effect was also obtained with ammonium hydroxide; not, however, with sodium hydroxide, which apparently kills the cell before it penetrates.

Although there is a great increase in the oil cap, chemical analyses indicate no change in the total lipid content of the egg, and the visible changes observed are apparently due to a release of bound lipid material. Ammonium salts are known to cause an alkalinization of the cell protoplasm and it is believed that the release of the lipid is due to this factor. This view is supported by the fact that when carbon dioxide is added to the medium, no increase in the volume of the oil cap occurs.

The eggs of the clam *Cumingia* behave like those of *Arbacia* when treated with ammonium salts, although in this instance the effect is not so striking. Very clear results may be obtained with *Amoeba proteus*. Thus when these organisms are exposed to dilute solutions of ammonium chloride and are then centrifuged after a few hours there is large accumulation of lipid at the centripetal pole of the cell, although control amœbæ show no such accumulation.

It is believed that in protoplasm generally alkalinization causes a release of bound lipid. The theoretical bearing of these observations will be discussed in the complete paper.

THE EFFECT OF CRUDE OIL POLLUTION ON MARINE LIFE. Paul S. Galtsoff.

August 27

DIFFUSION POTENTIALS IN BIOLOGICAL SYSTEMS. H. Burr Steinbach.

THE RÔLE OF IONS IN VALONIA AND IN NITELLA. W. J. V. Osterhout.

Experiments indicate that in these cells (as in many others) the protoplasmic surface consists of a liquid layer with a low dielectric constant. Kraus has shown that in such media charged complexes are formed. Hence if we place in the external solution K^+, we may have in the protoplasmic surface not only K^+ but also such charged complexes as $(KX_I)^+$, $(KX_{II})^+$, where X_I and X_{II} are elements or radicals.

Owing to the formation of such complexes the diffusion potential of KCl might seem to be much greater than that of NaCl in the protoplasmic surface. In consequence the calculated mobility of K^+ might appear to be much greater than that of Na^+. This may explain the following facts.

We find the following mobility ratios (taking the mobility of Cl^- as unity).

In water K ÷ Na = 0.99 ÷ 0.66 = 1.5
In *Nitella* K ÷ Na = 85.45 ÷ 2.18 = 39.2
In *Valonia* K ÷ Na = 20 ÷ 0.2 = 100

(likewise in muscle and in nerve the mobility ratio K ÷ Na may have a high value).

As such differences are not explainable by solvation or by the partition coefficients of simple ions, it would seem that they can be accounted for only by the formation of charged complexes.

Changes in such complexes might well be brought about either by removing substances from the protoplasmic surfaces or by adding them to it. This could explain the change of apparent mobilities from K > Na to Na > K in *Nitella* by treatment with distilled water,[1] and from K > Cl > Na to Na > Cl > K in *Valonia* by treatment with guaiacol (the cells are not injured by these two methods of treatment).

The Manner of Entrance of Ammonium Salts into Cells. M. H. Jacobs and Dorothy R. Stewart.

Ammonium salts of the type NH_4A, where HA is a weak acid, usually enter cells with ease; this behavior is plausibly accounted for by the presence, through hydrolysis, of NH_3 and undissociated HA, to both of which cells are, in general, permeable. Salts of the type NH_4Cl, which give rise to the single penetrating molecule NH_3, tend to make the interior of cells exposed to them more alkaline, but do not enter in sufficient quantities to cause appreciable osmotic volume changes except in the case of the erythrocyte which is permeable to anions. On adding a salt of the type BA to an isosmotic solution of NH_4Cl, the readily penetrating salt, NH_4A, is formed; this, however, does not distribute itself equally between the cell and its surroundings but according to the approximate equation:

$$[NH_4A]_{\text{inside}} = \sqrt{\frac{K_aK_b}{K_w}[NH_3]_{\text{outside}} \times [HA]_{\text{outside}}}.$$

Calculation shows that for the salts Na acetate and $NaHCO_3$ a considerable excess of internal osmotic pressure may be set up in this way; Na borate, on the other hand, is osmotically relatively ineffective. The behavior of the *Arbacia* egg is in good agreement with this theory. In solutions of NH_4Cl or KCl isotonic with sea water these eggs retain their original volumes. The addition of NH_4 acetate to KCl leads to a shrinkage followed by a return of the cell to its original volume; a similar addition of NH_4 acetate to NH_4Cl leads after an initial shrinkage to an increased final volume. Furthermore, while a considerably increased final volume results from the addition of Na acetate to NH_4Cl, the same is not true of Na borate. These results are believed to permit a more plausible explanation of the behavior of the erythrocyte in mixed solutions of ammonium salts than that of Ørskov which postulates a permeability of the cell to the NH_4^+ ion as such.

---

[1] Experiments in collaboration with S. E. Hill.

SOME ASPECTS OF ELEKTROLYTE DIFFUSION.* Torsten Teorell.

Based upon the classical theories of Nernst and of Planck a theory has previously been published (Teorell, *Proc. Nat. Acad. Sci.*, **21**, 152, 1935) dealing with the steady state conditions in regard to ionic distribution in aqueous systems where one elektrolyte was steadily diffusing, creating a diffusion potential. The concepts have now been extended in stating that any electrically charged particle, regardless of its size, is bound to be subjected to influence of diffusion potentials present in the system, positively charged particles being moved towards the negative part of the diffusion potential field and negative particles in the opposite direction.

Experimental results were demonstrated confirming the theoretical expectations. The technique used consisted in principle in maintenance of a steady diffusion gradient of a suitable electrolyte across a convection-proof boundary (a porous membrane: cellophane, "Membranfilter," filter paper) surrounded by two solutions containing the material under investigation. In several cases the conditions within the diffusion layer itself were investigated by means of the "multimembrane" arrangement (Teorell, *Science*, **81**, 491, 1935). Cases of accumulation and impoverishment were shown of inorganic ions, proteins, and colloidal suspensions. It was suggested that the technique could be used as a procedure for separation of substances in mixtures.

Finally it was emphasized that the "diffusion effect" upon ionic, colloidal etc. distribution, is not any electrolysis or cataphoresis effect, because no external E.M.F. is applied and no current is flowing. The diffusion effect is to be interpreted in terms of exchange of charged particles due to differences in mobility.

The bearings of the diffusion effects upon biological conditions were briefly mentioned.

August 29, 1935

STUDIES ON THE PHYSICAL PROPERTIES OF THE PLASMA MEMBRANE.

Robert Chambers.

The unfertilized mature egg of *Arbacia* can be rendered practically naked by washing in isotonic KCl and manipulating with microneedles. The surface of the egg is now occupied by a thin layer or film the integrity of which is essential for the life of the egg. Pressing the edge of the egg with the side of a horizontal needle against the coverslip may separate the film from the underlying granular cytoplasm, a clear zone of water-miscible fluid appearing between.

The film adheres to clean glass needles and is easily deformable. Strands dragged from it can be pinched off into spherical vesicles. Disrupting the film by tearing causes it to disappear whereupon cytolysis occurs. A fresh droplet of oil applied to the film snaps in making a flattened surface of contact on the inside of the film. An oil droplet allowed to remain for a few seconds in the sea water before touching the film simply adheres to it.

By pushing the egg in a shallow hanging drop the surface film can be made to flow. To demonstrate this two oil droplets are made to adhere to the surface, one being free and the other held by the delivery pipette. The egg is then pushed, upon which a churning movement occurs, the surface at the sides of the egg flowing forward while that on the top and bottom flow backward. The free, adhering droplet is carried along with the current while the other held by the pipette remains stationary although adherent to the streaming film. These experiments indicate that a thin film on the surface of protoplasm can be lifted off the underlying granular cytoplasm, it is fatty and it can be made to flow as a liquid.

---

* The details of this paper will be published in the *Jour. Gen. Physiol.*

MICROMANIPULATION OF EGG AND NURSE CELLS IN BOMBYX MORI. Hope Hibbard and Robert Chambers.

Cytoplasmic bridges connecting nurse cells with each other and with the egg of *Bombyx* have been demonstrated in sections of the maturing ovary. By the method of microdissection and microinjection, these can be shown in the live cell and substances can be made to pass through them. Dyes like phenol red injected into one cell of the group readily diffuse to all connected cells but not into adjacent cells to which there are no bridges. An oil drop forced into a nurse cell ran through the bridge into the egg and remained in a dumb-bell shape, part in the nurse cell, but the greater part in the egg, and the connection in the bridge. The consistency of the cytoplasm of the nurse cell is relatively much higher than that of its larger nucleus so that the tip of a pipette inserted into the cell tended to enter the nucleus. Under such a condition the injected oil filled and distended the nucleus without passing through the bridge to the egg cell. However, when the pipette lay in the cytoplasm, the oil ran readily on into the egg. On the other hand, an oil drop forced into an egg was increased in size until much larger than the previous one in the nurse cell, and yet it showed no tendency to pass through the bridge backward. The egg was much more easily distended and inflated than the nurse cell. This may be an explanation of the apparent "one-way valve" allowing material to pass from the nurse cell to egg cell but not backward, in the same direction the storage products normally pass from one to the other.

BUDDING OF CHLOROPLASTS AND EXTRA CHLOROPLAST FORMATION IN SPIROGYRA. Leonard B. Clark and E. E. Dale.

PARTHENOGENETIC MEROGONY OR CLEAVAGE WITHOUT NUCLEI IN ARBACIA PUNCTULATA. Ethel Browne Harvey.

The pigmented halves of *Arbacia* eggs, obtained by pulling apart the whole eggs by centrifugal force, serve as the material used in this study. Since the nucleus invariably goes to the lighter pole of the egg, the heavier red halves never have nuclei. Enucleate quarter eggs have also been used, obtained by further centrifuging of the red halves which pull apart into yolk quarters and pigment quarters.

When these enucleate halves and quarters are treated with parthenogenetic agents such as concentrated sea water, they throw off fertilization membranes. When transferred to sea water, cytasters appear in the eggs after about an hour, and after two or three hours cleavage planes come in. I have observed in many living fragments, especially in the yolk quarters, a large monaster, followed by an amphiaster, and this later by a cleavage plane between the two asters in quite typical form. By subsequent cleavages, three, four, five and many cells are formed, sometimes equal, sometimes unequal. Blastulæ of a hundred or more cells have developed from these non-nucleate fragments, quite normal in appearance, but as yet no swimmers.

The cleavages of these artificially activated enucleate halves and quarters resemble in every way (in the living material) those occurring in similar fertilized halves and quarters and in whole eggs (with nuclei) centrifuged and then fertilized, except that they occur much more slowly and there are no nuclei visible during the resting stages. They are also similar to those occurring in whole and half eggs, nucleated, activated by parthenogenetic agents.

Parthenogenetic merogony, or cleavage without nuclei, means, of course, that development may begin without either maternal or paternal chromosomes, and this involves fundamental problems of heredity and evolution.

THE QUANTITATIVE DETERMINATION OF MITOTIC ELONGATION. Leon Churney.

Curves have been plotted to show the course of mitotic elongation in the *Arbacia* egg at first cleavage. For eggs possessing fertilization membranes, the elongation is 11.5 per cent. When eggs are divested of their fertilization membranes, the elongation is 39.4 per cent. The data obtained are being used as a basis for further experimentation.

THE HYALINE ZONE OF THE CENTRIFUGED EGG OF NEREIS. Donald P. Costello.

CHANGES AT THE SURFACE OF ARBACIA PUNCTULATA EGGS DURING MEMBRANE ELEVATION. Floyd Moser.

A layer of small granules is imbedded in the inner margin of the cortical layer of *Arbacia punctulata* eggs. These granules resist movement under the influence of high centrifugal forces.

During the normal fertilization process, the cortical layer granules break down in wave-like fashion, beginning at the point of sperm entry and ending at the opposite side of the egg. An average of 8.5 seconds at 25.7° C. is required for the completion of the wave. Membrane elevation progressively follows the wave of granule breakdown.

When the light portion, or the light fragments of centrifuged eggs are slightly punctured with a microdissection needle, cortical layer granule breakdown occurs in wave-like fashion, followed immediately by membrane elevation around the light pole; membrane elevation follows at a relatively slow rate around the heavy pole. This membrane is the same as the fertilization membrane. Puncture of the yolk or pigment layers results only in local cytolysis.

Artificial parthenogenetic agents cause a change at the surface of centrifuged and uncentrifuged eggs similar to that caused by sperm entrance. In the presence of saponin, the "wave" and subsequent membrane elevation occur at the heavy pole first.

In all cases, breakdown of cortical layer granules is accompanied or followed by a disappearance of granules in the so-called fifth layer of centrifuged eggs. Following artificial stimulation, there is typically a cytolysis of this layer.

The above described phenomena do not take place in the absence of calcium. It is suggested that the reaction described is a surface precipitation reaction, involving a stimulus, the presence of calcium, the breakdown of granules, the subsequent release of the fertilization membrane and the establishment of a new membrane on the surface of the egg.

These observations are to serve as the basis of future experimentation.

LOCALIZATION OF PEPTIDASE ACTIVITY IN THE EGGS OF ECHINARACHNIUS AND ARBACIA. Heinz Holter.

Using the enzymatic micromethods developed by Linderstrøm-Lang and the author, an attempt was made to establish a quantitative correlation between the distribution of cytoplasmic constituents of eggs and their peptidase activity (Substrate Alanylglycine).

Arbacia eggs were stratified and separated into two halves by centrifuging, the halves containing different layers of the formed bodies (mitochondria, yolk, etc.). Ten eggs or egg-halves were used in each determination. It was found that the activity was unequally distributed, the centripetal half being 3–4 times as active as the centrifugal one. Since both halves showed enzymatic activity, all cytoplasmic constituents contained in one half only could be ruled out as bearers of the enzyme. Of the cytoplasmic constituents contained in both halves, only the hyaline plasma (matrix) showed a distribution similar to that of peptidase activity.

The volume of the hyaline plasma or matrix material was taken as equal to the total volume minus the volume of the formed bodies (measured by E. N. Harvey; in every case the ratio of matrix volumes for the two egg-halves agreed within 10 per cent with the corresponding ratio of peptidase activity.

The same relation holds true for the eggs of *Echinarachnius*. In this case, determinations could be made on single eggs or egg-parts. The egg-parts were secured by cutting stratified and elongated eggs on the micromanipulator. By this method it was possible to vary the distribution of egg constituents arbitrarily.

The ratio of total volumes for the upper and lower parts of the cut eggs was varied from 0.4 to 2.7, corresponding to a variation in the ratio of matrix volumes of 0.45–4.9. In every case the ratio of peptidase activities and of matrix volumes agreed within 10 per cent.

It is concluded, therefore, that peptidase is diffusely distributed, and is probably dissolved in the hyaline plasma or matrix of the egg cytoplasm.

EARLY STAGES OF OSSIFICATION. E. Alfred Wolf and Thomas F. McBride.

A modified silver nitrate method, developed by Gomori (1933), was used; it was successfully applied to whole embryos and heads of new-born rats. Sections of 10–15 microns were made which showed that the silver nitrate and the other reagents had evenly penetrated the tissues. The sections were stained in hæmatoxylin-eosin.

Loci of calcification can be recognized by the black silver that has replaced the calcium salts. In the vertebræ it was seen that calcification begins at the periphery of the cartilage, in a hyaline layer of fibroblast origin; but it is soon followed by the appearance of calcium deposits in the intercellular spaces of the cartilage, the first traces of which are seen there, where three cells touch. In the skull dermal and cartilagenous bones can be easily identified.

The method should be valuable for comparative anatomical and embryological studies. It is also intended to be used as a guide in micro-chemical phosphatase determinations of calcifying tissues.

EARLY STAGES OF CALCIFICATION IN TEETH. Thomas F. McBride and E. Alfred Wolf.

The Gomori silver nitrate method was used in the study of dentin and enamel formation in molars and incisors of embryos and new-born rats. The black deposits of silver that replace the calcium salts during the treatment of the tissues still permit the observation of structure in dentin and enamel under high power magnification (oil). The first deposition of lime salts occurs on that side of the predentin that borders on the ameloblast layer. The odontoblast cells are perfectly clear, with no traces of silver deposits. In the ameloblast layer the distal part of the cells that contains the nucleus is free of silver deposits; in the proximal part a gradual accumulation of silver can be seen, becoming very dense toward the end of the cells, forming here an apparently continuous black line running across the ameloblast cells. This line under high magnification resolves itself into individual dots—one for each cell. The upper part of the enamel itself can be seen to be composed of layers parallel to the surface that seem to be derived from the fusion of the intracellular dots of calcium salts mentioned before. The formation of enamel thus appears to be a rhythmical intracellular process.

OBSERVATIONS ON THE BEHAVIOR OF POLYMORPHONUCLEAR LEUCOCYTES AS SEEN IN THE LIVING ANIMAL. E. R. Clark, Eleanor Linton Clark, and R. O. Rex.

METHYLENE BLUE STAINING SEQUENCES IN THE WALLS OF THE DIGESTIVE TUBE OF THE FROG, RANA GRYLIO. Elbert C. Cole.

With methylene blue *intra vitam*, a fairly regular staining sequence was demonstrated in the muscle tunics of the intestine and rectum of the frog. When dye concentrations of about 1 : 5,000 in physiological saline were used, extraordinary specificity for nervous tissue was observed. Nervous tissue was first to show the stain, followed by muscle tissue and by connective tissue elements. There was also a sequence in the staining of various parts of a nerve cell. The cytoplasm of the cell body stained first. In the course of a few minutes the nucleus became distinct, and the irregular outline of the cell body became clearly differentiated. Following this, the axone stained heavily.

Although there was some variation in the twelve cases studied, and some overlapping of staining of the various cellular elements, the results were sufficiently regular to justify the assumption that sequence in staining is related to differences in the viability of the various cellular elements. The concentration of the dye, the nature of the solvent, the availability of oxygen, and the duration of staining are also factors concerned with this phenomenon.

THE EFFECTS OF ETHYL CARBAMATE AND OF POTASSIUM CYANIDE UPON THE STAINING CAPACITY OF GANGLION CELLS AND CELLS OF SMOOTH MUSCLE TYPE. Elbert C. Cole.

In methylene blue staining, *intra vitam*, the staining sequences observed were attributed to the relative viability of various types of cells in the body parts studied. Various substances were used to reduce the metabolic activity of cells. Ethyl carbamate, used in conjunction with methylene blue, gave strikingly selective preparations of the ganglion cells in the myenteric plexus of the frog, *Rana clamitans*. Potassium cyanide, on the other hand, gave a more general staining with methylene blue, and produced considerable distortion of cellular elements.

In the radial muscles of the squid chromatophore, ethyl carbamate and methylene blue gave irregular staining results. Potassium cyanide and methylene blue, however, produced excellent stained preparations of these cells, with but little cellular distortion.

These results give evidence that the capricious nature of methylene blue staining can be controlled to some extent, and that it is probably possible to secure selective staining of a considerable number of types of cellular elements by variations in the composition of the staining solution.

THE TOLERANCE OF ACETYL-SALICYLIC ACID BY SPERM AND EGGS OF ARBACIA. R. A. Budington.

The analgesic and antipyretic properties of acetyl-salicylic acid, as familiar to all in 'aspirin,' seem to produce the same effects as are known to follow the acidifying of sea water with other agents, so far as holding metabolism in suspension, and thus prolonging the vitality of freed *Arbacia* germ cells. In certain solutions, e.g. with pH 6.77, sperm were found to retain fertilizing powers for 75 hours, in contrast to a normal 7–20 hours; similarly, eggs retain fertilizability, followed by normal development after treatment for 57 hours. Development in solutions of pH 6.77 and 6.15 induces marked distortions of pluteus embryos.

THE SHEDDING REACTION OF ARBACIA PUNCTULATA. Louise Palmer.

When a sea urchin is cut or injured, it sheds eggs or sperm. This is apparently due to the release of a substance or substances from the injured tissues. Grinding the gonads or whole animals produces concentrations of tissue extract sufficient to induce shedding; higher concentrations are obtained by heating the ground animal with isotonic sodium chloride. The extract is not injured by heating at boiling for 10 minutes and is potent upon injection after being dialyzed or filtered. Its activity is antagonized by magnesium chloride. Spawning may be induced by (1) injecting the extract directly into the perivisceral cavity, (2) removing the animal from the water and placing a few drops on the aboral surface, (3) immersing the mature animal in a solution as low as 1 part extract to 75,000 parts of water sea. All latent periods are independent of the concentration and only upon injection is there a sex difference. Substances which cause shedding may also be obtained from *Fundulus* or frog muscle and from ground *Asterias*, *Echinarachnius*, or *Ostrea*.

Potassium chloride induces shedding by injection of as low a concentration as .005 M in isotonic sodium chloride and by immersion in 1 part potassium salt to 30 parts of sea water. Potassium is antagonized by magnesium chloride in the proportion of 1 to 7 for the isotonic solutions. Calcium chloride induces shedding by injection of at least .06 M and by immersion in one part calcium to seventy-five of sea water. One part isotonic calcium is antagonized by two parts magnesium chloride. Sodium chloride does not cause a reaction except in the absence of other salts, and magnesium salts never cause a reaction.

THE INDIVIDUAL SPECIFICITY OF BLOOD IN INHIBITING SELF-FERTILIZATION IN THREE SPECIES OF THE ASCIDIAN, STYELA. H. H. Plough.

SEX INVERSION IN TEREDO AND ITS RELATION TO SEX RATIOS. B. H. Grave and Jay Smith.

LIGHT REACTIONS AND PHOTORECEPTORS OF DOLICHOGLOSSUS KAWALEVSKYI. Walter N. Hess.

Like the earthworm, *Dolichoglossus* orients negatively to all ordinary intensities of light and positively to very weak light. If the thickening in the subepithelial plexus at the base of the proboscis is injured, no further orientations of the body occur, but the proboscis still has the ability to orient negatively to light.

A study of the photosensitivity of the different regions of the body by means of a pin-point light shows that the animal is sensitive to light over its entire body, the most sensitive regions being on the collar and at the tip of the proboscis.

As in the earthworm, *Dolichoglossus* has special cells at the base of the body epithelium that appear to be concerned with light reactions. Some of these cells are at the ends of nerve fibres, but most of them are connected with other similar cells, forming chains. These cells vary considerably in their morphology, yet they all resemble somewhat the rods and cones of the vertebrate eye, some being more like rods and some more like cones.

Since these special cells are more abundant in the regions having greater photosensitivity; since there are no other cells in these regions which stain with vital methylene blue which could be photosensitive; and since they seem to resemble in form the rods and cones of the vertebrate eye, it seems likely that these cells may be photoreceptor cells.

AN EXPERIMENTAL STUDY OF CLOT FORMATION IN THE PERIVISCERAL FLUID OF ARBACIA. James A. Donnellon.

The clot is due to cellular elements, and no clotting can occur after these are filtered off.

Tissue extracts of the shell or of any of the internal structures bring about clotting within a few seconds. These extracts are effective in dilutions of one to fifty. Extracts of *Limulus*, star-fish, crustaceans, molluscs, and shrimp produce the same effect, some being even more effective than *Arbacia* extracts. Extracts of frog muscle have no effect.

One or two cc. of isotonic KCl cause clot formation within a few seconds. It can be diluted one to four. An isotonic $MgCl_2$ solution will prevent clotting if added in sufficient quantity. If an equal amount of $MgCl_2$ is added to KCl or to tissue extract, these substances no longer bring about clot formation. $CaCl_2$ seems to hasten clotting although it is not so effective as KCl. LiCl and NaCl solutions have no observable effect.

Dilute solutions of fat solvents also cause clot formation.

A 10 per cent solution of peptone will inhibit clotting. A 1 per cent solution of sodium oxalate is only slightly inhibitory.

Solutions having a pH between 4.2 and 4.6 have a marked inhibitory effect on clot formation but do not prevent it entirely.

The addition of any foreign body will cause a clot to form around it.

If the peristomial membrane is cut a clot forms, closing in the opening. Similar clots have been observed in damaged parts of the intestinal wall. Scratching the inner wall of the test bring about a definite clot in that region.

Arbacia eggs broken up in ground glass or boiled also bring about clot formation. Unheated whole eggs have no effect.

The substance in the tissue extract responsible for the clotting effect will dialyze through a collodion membrane and is not destroyed by heat.

KEEPING NEREIS FOR PHYSIOLOGICAL STUDY. Manton Copeland.

Nereis virens in its natural environment is found principally in burrows in the sand or mud which are lined with mucus. If allowed to enter a glass tube open at both ends, which is placed in a shallow dish of sea water, it behaves essentially the same as it does in its burrows. Care should be taken to select a tube large enough to allow the animal to carry on characteristic undulatory movements of the body which draw the water through the tube. Moreover, the worm has the habit of turning frequently and its behavior is not normal if, on account of the too small diameter of the tube, it is unable to do this.

The sea water should be changed once or twice a week and the tube cleaned from time to time.

A constant diet of clam appears to keep the worm in a healthy condition. It requires only a few fragments (3 or 4 mm. in diameter) every day or two. These pieces of clam may be dropped at the end of the tube and juices emanating from them, when drawn into the tube, stimulate the animal to move forward to the food, which is immediately seized. It also will not hesitate to take the clam from forceps.

By this procedure it is possible to keep individuals in excellent condition for physiological study for long periods of time. Several have lived for over a year or two, and one for nearly three years.

My experience with *Nereis* has been confined to rather small animals, averaging about four or five inches in length.

SOME DIATOMS OF THE GREAT SALT LAKE AS INDICATORS OF PRESENT AND GEOLOGICAL WATER CONDITIONS.* Ruth Patrick.

This study of the diatoms of Great Salt Lake was undertaken in connection with the geological survey of Dr. A. J. Eardley of the University of Michigan. By means of a Musselman peat borer, samples were taken from various depths below the surface. Collections of algæ as well as surface sediments were also studied for the presence of diatoms.

The deepest sample, taken twenty feet below the surface, shows the presence of many fresh water diatoms. This supports the theory of the origin of the present Great Salt Lake by evaporation from the fresh waters of the former Lake Bonneville. Later deposits show the presence of euryhaline and brackish water forms which indicate the successive increase in the salt concentration of the water. The occurrence of fresh and brackish water diatoms in the craterlet muds of Rozell Point supports the theory of Eardley that swamps existed there in Pleistocene and Post-pleistocene times.

From the samples studied, it is interesting to note that a truly marine flora seems never to have established itself in the lake. In the examination of surface muds as well as in samples of living algæ I find no evidence of a diatom flora living in the lake proper today. This is to be expected in view of the fact that so few organisms can withstand the high salt concentration which exists in the lake.

THE ANATOMY AND HISTOLOGY OF THE ALIMENTARY TRACT OF THE GRASSHOPPER, MELANOPLUS DIFFERENTIALIS THOMAS.† Charles Hodge, IV.

This investigation is intended to be the first of a contemplated series to study correlation of diet with morphological change. The material here reported is from grasshoppers on satisfactory diets. The gross anatomy shows a typically acrididine arrangement. Fixation with B3 and staining in hæmotoxylin and eosin shows that peritoneum, muscle and chitin in different areas vary only in arrangement and relative development. The epithelial cells of the various regions differ in structure as well. Modifications of the cytoplasmic elements of the cells are correlated with cellular activity. The chitinous spines of the fore gut are secreted by cells temporarily provided with conical, cytoplasmic processes for the purpose. The development of the spines can be traced, probably for the reason that immediately after ecdysis the cells do not all secrete at the same rate. The peritrophic membrane originates from a groove in the epithelium of the fore gut at its insertion into the mid gut. The epithelial cells of the mid gut and its six bilobed gastric cæca have a striated border of separate filaments, which are cilia-like in appearance but devoid of movement. The cæca are most active in digestive secretion, which is merocrine. Essentially different granules and vacuoles are secreted, and extruded by rupture of the cells, which are replaced from adjacent nidi. The epithelial cells of the rectal glands have a striated border similar to that of the mid gut, but not so clearly and uniformly filamentous in structure.

CHANGES IN OSMOTIC PRESSURE OF TELEOST MUSCLE AS A RESULT OF CHANGES IN EXTERNAL SALT CONCENTRATION. Francis Leovey.

OSMOTIC EXCHANGES OF PHASCOLOSOMA. E. F. Adolph.

WATER CONTENT OF INSECTS IN RELATION TO TEMPERATURE AND HUMIDITY. W. A. Dreyer.

In preliminary experiments with the ant, *Formica exsectoides*, the body weight and total water content of workers was determined. Body weight varies from 6–17 mg. with 80.8 per cent of the individuals between 9 and 14 mg. The total water content of ants in all experiments varies from 64.8 to 81.6 per cent with a mean of 73.5 per cent. Active workers, at 22° C. and 70 per cent R. H., contain 66.9 to 81.6 per cent, mean 74.1 per cent. Hibernating workers, at 4° C. and 70 per cent R. H., contain 64.8 to 80.2 per cent, mean 72.8 per cent. There is no significant difference (Student's Method) in the total water content of active ants, hibernating ants, ants active in winter, or ants hibernating in summer. This insect maintains a relatively constant proportion of water in the tissues despite the modification of temperature and metabolic activity during hibernation. Experiments are being conducted with the flour beetle, *Tribolium confusum*, observing the relation of metabolic activity, as indicated by respiration, body weight, and water content, to different humidities at different temperatures. Thus far the range of total water content is found to be 69.8 to 83.3 per cent. No difference in total water content has been found for beetles at 24° or 10° C. and at 75 or 15 per cent R. H. The animals starving at the lower humidity lose more weight but maintain a constant proportion of water in the body, indicating a regulation of metabolism similar to that shown in the findings of Buxton and Mellanby for the meal-worm.

---

* Paper not delivered because of author's late arrival.

† Paper not presented because of author's late arrival.

AN ADAPTATION OF THE MANOMETRIC VAN SLYKE APPARATUS TO THE STUDY OF THE RESPIRATION OF MARINE ANIMALS. Millard W. Bosworth and William R. Amberson.

By slight modifications in the usual manometric Van Slyke technique a method has been worked out which has proven satisfactory for the determination of both O_2 consumption and CO_2 production of marine animals. The two gases can be determined on the same sample of water. The Van Slyke is equipped with a 100 cc. extraction chamber. Gas volumes may be read at 5 and 1 cc. A 30 cc. sample of water to be analyzed is drawn in a standard pipette with stop-cock, and handled in the usual manner. The solubility and resolution factors for carbon dioxide and oxygen must be determined empirically.

We have measured the gaseous exchanges of several fish and invertebrates common at Woods Hole. The animals are placed either in bicarbonate-free sea water or in normal sea water. Readings are somewhat easier in the former medium, which is adjusted to pH 8.2 by NaOH after removal of the bicarbonate by addition of acid and æration. The aquaria are sealed by placing a close-fitting fiber board disc covered with paraffin over the water. This effectively prevents exchange of gas with the air.

In bicarbonate-free sea water the pressure of CO_2 (read at 1 cc.) extracted from a 30 cc. sample at the beginning of the experiment runs between 10 and 30 mm. Hg. As CO_2 is produced this value rises to from 80 to 120 mm. Hg at the end. The O_2 gives 100 to 120 mm. Hg at the beginning, and falls to 10 to 20 mm. Hg at the end. A fairly sensitive determination of the respiratory gases is therefore possible.

A preliminary study of the flounder by this method shows that the O_2 consumption and CO_2 production are directly proportional to the oxygen tension in the sea water. The average R.Q. in nine experiments on five fish is .94 (.80 to 1.03).

THE RATE OF REGENERATION OF THE BLOOD PROTEINS AFTER COMPLETE EXSANGUINATION. John Stanbury and William R. Amberson.

The work of Whipple and his co-workers indicates that the blood proteins cannot be depleted below one gram of protein per hundred grams of plasma without the appearance of fatal shock and he concludes from this fact that the plasma proteins make up a specific and essential part of the environmental complex of the body cells. He finds that the plasma proteins regenerate to a 6 per cent normal from a depletion level of one per cent in from five to fourteen days. Our technic is to perfuse cats with a suspension of erythrocytes in 6 per cent Lilly gum acacia and ringer-locke through the carotid artery. We have used erythrocytes from the ox, the duck, and cats. Cats perfused with ox cells behave in an approximately normal fashion, but the cells slowly hemolyze and the animal dies in three or four days because of lack of hemoglobin. In these animals we noted a marked overshooting above the normal of the plasma proteins. Cats perfused with duck cell-acacia-ringer-locke lived for only about two hours. Cats perfused with cat cell-acacia-ringer-locke, while more nearly normal in some respects than ox cell perfused cats, show a central nervous disorder in some cases which finds its possible explanation in the fact that acacia agglutinates cat cells. These agglutinations may form blocks within the central nervous system. By our technic we can carry the blood proteins down to 0.1 gram of protein per hundred cc. of plasma without the appearance of shock. The blood pressure, temperature, respiratory rate, and heart beat show no marked alterations. From this we conclude that the chief function of the plasma proteins is the maintenance of the colloidal osmotic pressure of the blood and that the plasma proteins do not form a specific, but only a physical necessity to the body cells We also find that the plasma proteins in cats which have had their plasma proteins depleted to 0.1 per cent regenerate to normal in four to four and one half days. There is a very rapid early rise due undoubtedly to an influx of extravascular protein and a slower subsequent rise.

The Permeability of the Frog Liver to Certain Lipoid Insoluble Substances. Charlotte Haywood and Rudolf Höber.

The permeability of the secreting cells of the liver to several lipoid insoluble substances of varying molecular weight has been investigated by perfusing the isolated liver of the bullfrog with oxygenated Ringer's or Brömser's solutions containing these substances. The addition of the dye, eriocyanin, to the perfusion fluid was routinely employed as a criterion for the survival of the liver, since the active liver is known to concentrate dyes markedly. Perfusion pressures of 6–8 cm. H_2O were used in the later experiments. Secretion for analysis was collected in a cannula tied into the neck of the gall bladder, close to the cystic ducts. The Shaffer-Hartmann-Somogyi micromethod for reducing sugar, the Somogyi method for fermentable sugar, and the Hirschfelder-Serles method for magnesium were used in analysis.

Even when the perfusion fluid contained no sugar, more or less fermentable reducing substance appeared in the secretion. The addition of xylose, lactose, or inulin to the perfusion fluid resulted in the reappearance in the secreted fluid of similar concentrations of these substances. The passage of soluble starch into the secretion could not be detected by the iodine test. It therefore appears that the entrance of lipoid insoluble substances into the bile is limited to those substances having a molecular weight or aggregation somewhere between that of inulin and that of soluble starch.

Experiments in which magnesium chloride was added to the perfusion fluid showed that the magnesium ion also passes readily into the secretion without concentration.

The fact that the various substances investigated were in no way concentrated by the liver is in sharp contrast to the liver's concentrating action upon dyes and suggests that different mechanisms may be concerned in the two cases.

Effect of Nitrophenols and Related Compounds on Metabolism of Living Cells. M. E. Krahl and G. H. A. Clowes.

A comparison between the chemical and biological properties of substances known to act as reversible oxidation-reduction systems and the nitrophenols and their derivatives shows, among others, the following significant differences: (1) 4, 6 dinitro-o-cresol and other analogous substances have their optimum concentration for stimulation at about 5×10^{-6} molar as compared to the range 5×10^{-4} to 5×10^{-3} molar for a series of redox substances. (2) At concentrations up to one hundred times the oxidation optimum the nitro compounds are not irreversibly toxic to the cell; with all redox substances studied the cells are killed by concentrations which give a rise in oxygen consumption. (3) From all the literature available the nitro and dinitrophenols are incapable of reversible oxidation reduction in the range of potentials characteristic of the living cell. (4) The partial reductants of p-nitrophenol, such as p-nitrosophenol, do not, in low concentrations, raise oxygen consumption or block division reversibly. (5) The optimum concentration for raising respiration or blocking division of any given dinitro compound is not significantly affected by raising or lowering the temperature of the medium, indicating that the nitro compound is not undergoing a reaction with a velocity sensitive to temperature, but is more likely to be concerned in a process which is relatively insensitive to temperature.

Evidence that the oxidation-promoting effect is probably not due to stochiometric reduction of the nitro compound by constituents of the cell is as follows: (1) The effect of a given concentration of nitro compound is independent of the number of cells within a wide range. (2) Concentrations, similar to those found effective for the nitrophenols, of substances easily reduced by the cell have little or no effect on respiration. (3) The nitro group or groups may be left in the molecule, while other groups are substituted for the OH, without retaining the effect of the nitrophenols on oxidation and division.

STIMULATION AND DEPRESSION OF RESPIRATION IN RELATION TO CELL DIVISION. G. H. A. Clowes, M. E. Krahl and A. K. Keltch.

The observation reported last year that certain nitro compounds, particularly 4, 6 dinitro-o-cresol, suppressed division of fertilized eggs of *Arbacia* at the peak of respiration, and that the block to division occurred in the prophase of mitosis, has been confirmed. Dinitrocresol exerts a similar block to division at the peak of respiration in eggs of other species as follows: *Echinarachnius*, prophase; *Asterias*, all phases; *Cumingia* and *Nereis*, metaphase. These blocks to division are generally reversible over a wide range of concentration.

Further support of the conclusion that the block to division occurring at the peak of respiration is not due to the increase in oxygen consumption is found in the following observations: (1) When the oxygen tension is gradually lowered the effect of dinitrocresol on respiration is gradually diminished until finally little or no effect is exerted, but the reversible block to division is exhibited throughout at the same concentration at which it occurs in a normal oxygen atmosphere. (2) While dinitrocarvacrol exerts an effect on respiration and cell division which is almost identical with that of dinitrocresol, its isomer, dinitrothymol, exerts practically no effect on respiration but still causes a highly effective reversible block to division at dilutions even greater than those at which dinitrocresol is effective.

Paranitrophenol and metanitrophenol exert effects on respiration and cell division similar to those of dinitrocresol in a slightly higher concentration range. Orthonitrophenol exerts no effect on either respiration or cell division at these concentrations but only a slight effect on respiration at high concentrations at which the eggs are irreversibly injured. Trinitrophenol and p-nitrodimethylaniline, which has a dipole moment as large as that of paranitrophenol, exert no effect on respiration or division in the low concentration range.

The above facts, particularly the difference in behavior of the ortho, meta and paranitrophenols, lend further support to the view that the action of nitrophenols on respiration and cell division may be attributable to their property of forming addition compounds with other chemical entities and adsorbing on interfaces.

The fact that nitro compounds have been found to stimulate anerobic fermentation in living yeast but not in cell-free systems seems to support the view that nitro compounds may function by blocking side reactions, thus diverting more substrate into the channels of oxidation, rather than by stimulating one of the oxidation steps.

INDOPHENOL OXIDASE ACTIVITY IN INTACT AND FRAGMENTED ARBACIA EGGS. Albert E. Navez and Ethel Browne Harvey.

The problem of determining the importance of the nucleus as a controlling agent in metabolism of cells has received a great deal of attention in the past, but technical difficulties have prevented to a great extent the drawing of conclusions resting on secure bases.

The centrifuge technique developed by E. N. Harvey and E. B. Harvey enables one to obtain easily "half eggs"; nucleated (colorless half) and enucleated (pigmented half), each one viable and with all the potentialities of the whole egg.

Recent work by H. Shapiro has brought to light remarkable differences in O_2 consumption for the two halves, the enucleated one having a much larger consumption (by nearly 90 per cent) than the nucleated one. These results strengthened the idea that the echinochrome is probably playing an important rôle in the oxygen transfer.

In order to see if oxidase activity is determining this type of difference in oxygen consumption, experiments were made, using the nadi reaction, to estimate the indophenoloxidase activity of normal eggs (used as a standard), of stretched eggs (centrifuged) and of the two halves of eggs split by centrifuging. A few determinations were done also on fertilized eggs. When all measurements are reduced to equal volumes of cells or parts of cells (taken before centrifuging) the relative values found are as follows:

Whole eggs unfertilized	1.0	Whole eggs fertilized	1.4
"White" halves (with nucleus)	2.4	Whole eggs stretched	3.2
"Red" halves (with echinochrome)	4.8		

On the bases of relative volumes these figures are of the same order of magnitude and sign as the ones found for oxygen consumption. Cytolysis of the eggs before addition to the nadi increases slightly these figures. Interpretation of these values is to be done in terms of surface, volume, and membrane properties.

DIFFERENTIAL OXYGEN UPTAKE OF REGIONS OF LIMULUS OPTIC NERVE AS RELATED TO DISTANCE FROM THE SENSE ORGAN. Rita Guttman.

The optic nerve of the king-crab, *Limulus polyphemus*, was cut into five parts and the oxygen uptake of each part determined with the Gerard-Hartline capillary microrespirometer, a form of differential volumeter (*Jour. Cell. Comp. Physiol.*, **4**, 141, 1934). In eleven preliminary experiments performed, the region most distal to the central nervous system was invariably lower in respiratory rate than the most proximal region. A maximal respiratory rate usually occurred in intermediate regions and thus there is no oxygen consumption gradient in the accepted sense along this nerve. The average difference between the maximal rate and the rate at the periphery was 37 per cent. These relationships were maintained through a temperature range of 16° to 31° C.

The results do not agree with those obtained by Tashiro (*Jour. Biol. Chem.*, **18**, 329, 1914) in his study of CO_2 production by *Limulus* optic nerve (assuming R.Q. = .8), both as regards relative and absolute rates. This may be accounted for by the fact that Tashiro measured the nerve's activity shortly after dissection when the rate is of variable magnitude and supernormal owing to stimulation by dissection and cutting. The respiration measured here was the subsequent uniform rate, which was maintained in these experiments through a period of about five or six hours.

It is interesting to compare the relative respiratory rates obtained in these experiments with the graduations in velocity along the fastest fibers of the green frog sciatic as measured by Marshall and Gerard (*Amer. Jour. Physiol.*, **104**, 586, 1933). These authors found that the velocity increases from the proximal to the middle portion, and then decreases peripherally.

LACTIC ACID IN DOGFISH NERVE. Walter S. Root.

MECHANICAL PROPERTIES OF SMOOTH MUSCLE. Emil Bozler.

A load is suddenly applied to the tonus muscle of *Pecten* and the extension time curve recorded optically. The muscle is at first extended suddenly by the length Δl. Then it continues lengthening slowly at a constant rate. This phenomenon is discussed under the assumption that the contractile elements are of the nature of an elastic liquid. The equation $dl/dt = \Delta l/Z$ is derived for the extension time curve (Z is the "relaxation time," the time in which the tension decreases by $1/e$ at constant length). The shape of the observed extension time curve agrees with the calculated curve. Furthermore, the absolute value of Z as calculated from these curves agrees within the limits of the experimental error with the value found by direct measurement. It is concluded that the conception of the contractile elements being of the nature of an elastic liquid gives a quantitative explanation of the mechanical properties of smooth muscle both at constant length (as shown in *Zeitschr. vergl. Phys.*, **14**, 1931) but also at constant external force.

A METHOD FOR MEASURING THE INTERCELLULAR SPACES IN TISSUES. A. K. Parpart.

SPATIAL RELATIONS IN THE EXCITATION OF THE ISOLATED MUSCLE FIBRE. F. J. M. Sichel and C. Ladd Prosser.

To investigate the possibility of spatial summation and the effects of varying cathode area on the non-conducted twitch response of the isolated skeletal muscle fibre, two separate small cathodes and a single, very large, anode were used. These were all silver-silver-chloride electrodes. The two cathodes were arranged in parallel in the electrical circuit with suitable keys so that the fibre could be stimulated by each one separately or by both simultaneously. The stimuli were short- duration (8σ) direct-current shocks.

The fibre was dissected from the adductor magnus of the bullfrog and mounted for recording isometrically in a manner described previously by one of us.

On stimulation with both cathodes simultaneously, the fibre was found to develop a tension at least equal to the sum of the tensions developed by each cathode when it alone is in the circuit. Frequently the fibre would respond to both cathodes when each alone was subthreshold.

The tension produced by simultaneous stimulation with both cathodes, relative to the sum of the tensions produced by each separately, tends to decrease as the voltage (and consequently, the response) increases. It also decreases as the distance between the cathodes increases, so that ultimately the ratio of the response to both cathodes simultaneously to the sum of each separately approaches unity.

We offer the following qualitative explanation of these results in terms of the electrical field:

1. If the p.d. across the fibre due to each cathode is plotted as a function of the distance along the fibre, a curve is obtained with a maximum opposite the position of the electrode.

2. The fibre will develop a tension which is a function of the area under this curve above the threshold value.

3. Since p.d.'s may be added, the curve for both electrodes may be obtained by adding the ordinates due to each electrode alone.

4. It will be seen that when the cathodes approach one another the area above the threshold and under the compound curve increases. The effects of varying the voltage and the results found when one or both cathodes used alone is subthreshold may be similarly explained.

The Permeability of the Erythrocyte to Ammonia. M. H. Jacobs and A. K. Parpart.

In a recent paper, Ørskov opposes the theory that in the penetration of the erythrocyte by ammonium salts NH_3 molecules rather than NH_4^+ ions are involved on the ground that osmotic swelling in solutions of ammonium salts is decreased at alkaline reactions where the concentration of NH_3 is increased. This argument, however, fails to take into account the facts (1) that the entrance of an ammonium salt requires not only the penetration of NH_3 but also either an exchange of OH^- ions for anions of the penetrating salt or the simultaneous entrance of undissociated acid molecules, both of these processes being hindered by alkalinity of the external medium, and (2) that by changes in the manner in which base is combined within the cell, external alkalinity tends to decrease the osmotic pressure of the latter and so to favor shrinkage. In the present investigation, positive evidence of the rapid penetration of NH_3 is given by comparing the volume changes that occur when NaOH, on the one hand, and NH_4OH, on the other, are added to suspensions of erythrocytes in isotonic NaCl. In both cases shrinkage of the cells occurs during periods of several minutes, but in the latter case alone this shrinkage is preceded by an initial swelling which appears within a few seconds. Such a swelling would be demanded, theoretically, if the penetration of NH_3 were rapid compared with the ionic exchange that leads to the final equilibrium, but would be difficult to account for, if, as Ørskov believes, NH_3 entered the cell slowly. Swelling curves of erythrocytes in solutions of NH_4Cl alone and after the addition of NH_4OH and HCl further confirm the view that NH_3 penetrates the erythrocyte with ease.

The Effect of Lack of Oxygen on the Permeability of the Egg of Arbacia punctulata to Ethylene Glycol. F. R. Hunter and E. N. Harvey.

In previous experiments (Kekwick and Harvey, *Jour. Cell. and Comp. Physiol.*, 5: 43–51, 1934) it was found that water penetrated the egg of *Arbacia* more rapidly under ærobic conditions than under anærobic conditions. The present experiments, however, indicate that ethylene glycol penetrates more rapidly under anærobic conditions. Eggs placed in an isosmotic solution of 0.5 molar ethylene glycol and 50 per cent sea water appear to swell more rapidly under anærobic conditions. Eggs placed in a hypertonic solution of ethylene glycol in normal sea water appear to attain their minimum volume sooner under anærobic conditions and also, there appears to be a greater decrease in volume than when oxygen is present. As the eggs swell after having attained their minimum volume, the rate is more rapid in the absence of oxygen. In both sets of experiments, although the rate of penetration of ethylene glycol appears to be affected by anærobic conditions the final equilibrium is not affected.

BIOLOGICAL MATERIALS

For forty-five years the Marine Biological Laboratory Supply Department has been furnishing both living and preserved specimens to schools, colleges, and experimental laboratories. It is the desire of the Laboratory to continue this service in an efficient and satisfactory manner, and your co-operation is earnestly desired.

The fauna and flora are freshly collected each season and this gives assurance that materials purchased from this station are in the finest condition. Only the best selection of specimens are prepared and stocked and the suggestions of our customers regarding transportation and preparation are welcomed. Shipments are guaranteed, and if there should be any dissatisfaction, the purchaser is urged to notify us, that satisfactory adjustment may be made.

At this time especially, the Supply Department wishes to remind you of the shipments of living Marine Aquaria Sets. For several years now these well-balanced sets have gone forward to customers in North Dakota, Iowa, New Mexico, and Texas; as well as to many other states and Canada. This service has proven to be of great value to both instructor and student, particularly to those who are located at some distance from the sea coast. Detailed information and instructions regarding these shipments will be gladly sent upon request, and shipments are made from November through February with the guarantee that they will ship in good condition as far west as the Mississippi and as far south as Georgia.

We would appreciate the opportunity to take care of your needs for Biological materials. The Supply Department is always ready to serve you, and orders are usually shipped within 24 hours of their receipt unless a definite shipping date is specified.

1935 Catalogue on request

SUPPLY DEPARTMENT
Est. 1890

MARINE BIOLOGICAL LABORATORY
WOODS HOLE, MASS.

CONTENTS

Page

ROEDER, K. D.
An Experimental Analysis of the Sexual Behavior of the Praying Mantis (Mantis religiosa L.) 203

BRAND, THEODOR V.
Methods for the Determination of Nitrogen and Carbon in Small Amounts of Plankton 221

SAYLES, LEONARD P.
The Effects of Salinity Changes on Body Weight and Survival of Nereis virens 233

GRAFFLIN, ALLAN L.
Chloride and Total Osmotic Pressure in the Blood of Marine Teleosts 245

BARNES, T. CUNLIFFE
Salt Requirements and Orientation of Ligia in Bermuda, III 259

TURNER, C. L.
Studies on the Secondary Sexual Characters of Crayfishes: X. The annulus ventralis in true intersexes of Cambarus . . . 269

HEILBRUNN, L. V., AND R. A. YOUNG
Indirect Effects of Radiation on Sea Urchin Eggs 274

HEILBRUNN, L. V., AND KATHRYN DAUGHERTY
Indirect Effects of Radiation on Elodea Leaves 279

HARVEY, ETHEL BROWNE
The Mitotic Figure and Cleavage Plane in the Egg of Parechinus microtuberculatus, as Influenced by Centrifugal Force . . 287

HARVEY, ETHEL BROWNE
Some Surface Phenomena in the Fertilized Sea Urchin Egg Influenced by Centrifugal Force 298

HAIG, CHARLES
The Phototropic Responses of Avena in Relation to Intensity and Wave-length 305

PROGRAM AND ABSTRACTS OF SCIENTIFIC PAPERS
Presented at the Marine Biological Laboratory 325

Volume LXIX Number 3

THE
BIOLOGICAL BULLETIN

PUBLISHED BY
THE MARINE BIOLOGICAL LABORATORY

SONIAN INSTITUTION
DEC 11 1935
NATIONAL MUSEUM

Editorial Board

GARY N. CALKINS, Columbia University
E. G. CONKLIN, Princeton University
E. N. HARVEY, Princeton University
SELIG HECHT, Columbia University
LEIGH HOADLEY, Harvard University
M. H. JACOBS, University of Pennsylvania
H. S. JENNINGS, Johns Hopkins University
E. E. JUST, Howard University
FRANK R. LILLIE, University of Chicago
CARL R. MOORE, University of Chicago
GEORGE T. MOORE, Missouri Botanical Garden
T. H. MORGAN, California Institute of Technology
G. H. PARKER, Harvard University
W. M. WHEELER, Harvard University
EDMUND B. WILSON, Columbia University

ALFRED C. REDFIELD, Harvard University
Managing Editor

DECEMBER, 1935

Printed and Issued by
LANCASTER PRESS, Inc.
PRINCE & LEMON STS.
LANCASTER, PA.

THE BIOLOGICAL BULLETIN

THE BIOLOGICAL BULLETIN is issued six times a year. Single numbers, $1.75. Subscription per volume (3 numbers), $4.50.

Subscriptions and other matter should be addressed to the Biological Bulletin, Prince and Lemon Streets, Lancaster, Pa. Agent for Great Britain: Wheldon & Wesley, Limited, 2, 3 and 4 Arthur Street, New Oxford Street, London, W.C. 2.

Communications relative to manuscripts should be sent to the Managing Editor, Marine Biological Laboratory, Woods Hole, Mass., between June 1 and October 1 and to the Institute of Biology, Divinity Avenue, Cambridge, Mass., during the remainder of the year.

INSTRUCTIONS TO AUTHORS

Preparation of Manuscript. In addition to the text matter, manuscripts should include a running page head of not more than thirty-five letters. Footnotes, tables, and legends for figures should be typed on separate sheets.

Preparation of Figures. The dimensions of the printed page (4⅜ x 7 inches) should be borne in mind in preparing figures for publication. Drawings and photographs, as well as any lettering upon them, should be large enough to remain clear and legible upon reduction to page size. Illustrations should be planned for sufficient reduction to permit legends to be set below them. In so far as possible, explanatory matter should be included in the legends, not lettered on the figures. Statements of magnification should take into account the amount of reduction necessary. Figures will be reproduced as line cuts or halftones. Figures intended for reproduction as line cuts should be drawn in India ink on white paper or blue-lined coördinate paper. Blue ink will not show in reproduction, so that all guide lines, letters, etc. must be in India ink. Figures intended for reproduction as halftone plates should be grouped with as little waste space as possible. Drawings and lettering for plates should be made directly on heavy Bristol board, not pasted on, as the outlines of pasted letters or drawings appear in the reproduction unless removed by an expensive process. Methods of reproduction not regularly employed by the Biological Bulletin will be used only at the author's expense. The originals of illustrations will not be returned except by special request.

Directions for Mailing. Manuscripts and illustrations should be packed flat between stiff cardboards. Large charts and graphs may be rolled and sent in a mailing tube.

Reprints. Authors will be furnished, free of charge, one hundred reprints without covers. Additional copies may be obtained at cost.

Proof. Page proof will be furnished only upon special request. When cross-references are made in the text, the material referred to should be marked clearly on the galley proof in order that the proper page numbers may be supplied.

Entered October 10, 1902, at Lancaster, Pa., as second-class matter under Act of Congress of July 16, 1894.

Vol. LXIX, No. 3 December, 1935

THE

BIOLOGICAL BULLETIN

PUBLISHED BY THE MARINE BIOLOGICAL LABORATORY

ACID FORMATION IN FROZEN AND THAWED ARBACIA PUNCTULATA EGGS AND ITS POSSIBLE BEARING ON THE PROBLEM OF ACTIVATION

JOHN RUNNSTRÖM[1]

(From the Institute of Experimental Zoölogy, Stockholm, Sweden, and the Marine Biological Laboratory, Woods Hole)

According to observations reported by E. B. Harvey (1930) and the writer (Runnström, 1930) the sea urchin egg can be fertilized under anaerobic conditions. The further development, however, even the fusion of the pronuclei, is blocked. The writer (Runnström, 1930, 1933) furthermore stated that an acid is formed following aerobic as well as anaerobic fertilization of the *Paracentrotus* egg. The quantity of acid formed was found by Warburg's manometric methods to be equal to about 150 cu. mm. per cc. of egg substance. Under anaerobic conditions the acid formation seems to be 20–25 per cent higher. Acid formation also takes place after treatment of the eggs with hypertonic solutions. Higher values were found under anaerobic than under aerobic conditions. Acid formation is also increased in the *Paracentrotus* egg with increasing concentrations of the hypertonic solutions. The acid formation does not correspond to an increase in lactic acid formation nor to an increase of the content of inorganic phosphate (Runnström, 1933). Örström (1935) found that the formaline titration did not reveal any increase in the amino-nitrogen content following fertilization of the *Paracentrotus* egg. The same author (1935) also found that ammonia is formed at a slow rate in the unfertilized *Paracentrotus* eggs. Upon activation by sperm or by a hypertonic solution there is a marked increase of ammonia production, corresponding to about 0.25 cc. n/100 NH_3 per 1 cc. of egg substance. After the outburst following activation ammonia production stops in the fertilized egg. In spite of the formation of ammonia in the activation process the egg suspension gets more acid due to the simultaneous formation of an acid.[2]

---

[1] Fellow of The Rockefeller Foundation, 1933–34.

[2] In the course of further experiments Örström arrived at the conclusion that not free ammonia but a compound containing ammonia is formed upon fertilization. Ammonia is split off from this compound on applying the Parnus procedure for ammonia estimation.

In a previous paper (Runnström, 1933) it was stated that a breaking up of the *Paracentrotus* eggs in distilled water is accompanied by a strong acid formation. The further study of the acid formation in the broken up eggs seemed of interest in view of its possible bearing on the activation process. The observations were carried out at the Marine Biological Laboratory, Woods Hole, with the eggs of *Arbacia punctulata.* The work was also regarded as a preparation for further attempts towards the chemical identification of the acid formed.

Dr. Alfred Mirsky suggested to the writer a method of breaking up the eggs which is more appropriate than the treatment with distilled water and has great advantages also for the determination of acid formation. The eggs are frozen at about — 80° in carbon dioxide snow and ether. After a longer or shorter time the eggs are thawed at room temperature. Here the influence of different substances on the remaining metabolism can be tried without interference by the semipermeability of the egg surface. The acid formation was measured by the Warburg manometric method. Three cc. of egg suspension were transferred to each vessel; one side-arm contained 0.3 cc. 2–n H_2SO_4. The vessels were carefully stoppered and immersed into the mixture of ether and dry snow. After some time the vessels were removed from this mixture and the suspension thawed at room temperature. The vessels were joined to the manometers after one hour of thawing. After establishment of the temperature and gas equilibrium, sulfuric acid was tipped into the main compartment of the vessel. The manometric readings give the content of combined carbonic acid in the thawed egg suspension (B II). The combined carbonic acid was determined in the same way in a non-frozen portion of the egg suspension (B I). The difference between the two determinations of the combined carbonic acid $\Delta B = B\,I - B\,II$ is proportional to the acid formation. Samples of sea water were treated in the same way as the egg suspensions but no significant differences were found in this case. The value obtained for ΔB should be multiplied by a " retention factor " which, however, has not been determined for this material. The values given below are uncorrected and it is to be remembered that they are throughout lower than the real values.

In one experiment, July 25, 1935, B I was found to be 127 and B II 21 cu. mm., ΔB is then 106 cu. mm. The concentration of the suspension was 7.5 vol. per cent; this means about 470 cu. mm. acid per 1 cc. egg substance. In a parallel experiment to this the egg suspension contained 0.035 m sodium monoiodoacetate. In this case B I was 125 cu. mm., B II 20.5 cu. mm., ΔB is thus 104.5 cu. mm. The agreement between the two ΔB values shows that iodoacetate has no influence on the acid formation.

In one experiment, July 26, 1934, B I was 131 cu. mm. and B II 19.5 cu. mm. ΔB is thus 116.5 cu. mm. As the suspension was 8.6 vol. per cent this means 450 cu. mm. per 1 cc. of egg substance. In a parallel experiment the egg suspension contained 0.0004 per cent $CuCl_2$. B I was 132, B II 13 cu. mm., ΔB = 119 cu. mm. It is evident that copper does not inhibit the acid formation.

In the same way it has been shown that 0.06 m sodium fluoride has no influence on the acid formation in the thawing eggs. The addition of 0.008 per cent pyocyanine was likewise without any effect.

In one experiment, July 27, 1934, the influence of hexosemonophosphate was tested. To 3 cc. egg suspension 0.15 cc. 0.25 M hexosemonophosphate was added (Robison ester). ΔB was here 90 cu. mm., while it was 88 cu. mm. without addition of hexosemonophosphate. In a parallel experiment with hexosemonophosphate the egg suspension contained also 0.02 M iodoacetate. ΔB was here 98 cu. mm. The suspension was 16 vol. per cent. The figures tend to show that hexosephosphate has no essential influence on the acid formation. The retention factor may be somewhat higher for the mixture containing hexosephosphate, cf. the analogous case with the eggs cytolyzed in distilled water (Runnström, 1933, p. 276). If the acid formation were a glycolysis enhanced by addition of hexosephosphate, the acid formation should be decreased by addition of iodoacetate which inhibits the oxidoreductions involved in glycolysis. Even in suspensions with hexosephosphate added the acid formation is the same either with or without iodoacetate.

An acid formation took place also in thawing fertilized eggs. This is about equal to that found in unfertilized eggs. In one experiment (August 1, 1934) ΔB was found to be 117 cu. mm. for unfertilized and 107.5 cu. mm. for fertilized eggs. The suspension was 10 vol. per cent.

In some of the later experiments the suspension of thawed eggs was centrifuged, the solid parts collected as a pulp on the bottom of the tubes, separated from a colored fluid. The combined carbonic acid was determined in the fluid part. Very low values were now found as compared with the values obtained with the uncentrifuged thawed eggs.

In one experiment (August 18, 1934) the eggs were frozen and kept so for two days, then thawed. B I was 278 cu. mm., B II 144 cu. mm., ΔB is then 134 cu. mm. A portion of the thawed eggs was centrifuged. After this B II was found to have the value of 48 cu. mm. and ΔB is thus 230 cu. mm. This curious fact may be explained by assuming that some proteins centrifuged down in the quoted experiment acquire a CO_2-binding capacity during the acid formation which may bring them to the acid side of their isoelectric point. Since, in every experiment, the different samples to be compared were treated in exactly the same manner, the reported result will not annihilate the conclusions drawn above from experiments with uncentrifuged thawed eggs. As pointed out already, the figures given have only relative value.

The thawed eggs show an oxygen uptake, which is very much enhanced by the addition of pyocyanine or methylene blue. The oxygen uptake is still more increased on addition of hexosephosphate (cf. Runnström, 1935); 0.03 m iodoacetate or 0.06 m fluoride inhibit the respiration enhanced by hexosephosphate 30–35 per cent.

Unfertilized eggs were transferred into the fluid extract remaining after centrifuging off the solid particles from thawed eggs. After some few minutes a number of the eggs are surrounded by an easily visible, distinct jelly. Probably the visible jelly is a coagulation product of the jelly surrounding the egg. This jelly in general can only be demonstrated by the addition of certain stains or India ink. The neutralized extract exhibited the same influence on the jelly. Heating the extracts for 10 minutes to 70° does not remove the action on the jelly. In the course of the present work an acid formation amounting to 150–170 cu. mm.[3] per 1 cc. of egg substance was found to follow fertilization also in *Arbacia*. A similar acid production takes place upon aerobic treatment of the unfertilized *Arbacia* eggs with a stronger hypertonic solution according to Just (1922). The question arises as to whether the acid formation in the thawing eggs has the same chemical basis as the acid formation following the activation of the egg. This cannot be decided as yet but seems very likely. In the *Paracentrotus* egg a gradual increase of acid formation was found with increasing concentration of the hypertonic solution (cf. above). Finally concentrations are reached in which the eggs are cytolyzed. It must be pointed out that after cytolysis the acid formation stops, both in the eggs treated by a hypertonic solution and in the thawed eggs. The acid formation described above is perhaps identical with the "acid of injury" sometimes referred to.

The inhibition experiments reported above allow certain negative conclusions as to the chemical nature of the acid formed in the broken up eggs. Lactic acid formation is excluded by the iodoacetate experiments, as pointed out already above. The phosphorylation of hexoses is also inhibited by iodoacetate (Lundsgaard, 1930), the dephosphorylation of phosphoglyceric acid by fluoride (Embden and Deuticke, 1935).

The experiments mentioned above on the inhibition of the oxidations of hexosephosphate in the system thawed eggs + pyocyanine by iodoacetate and fluoride prove that these compounds interfere with the carbohydrate metabolism of the eggs. Also in dry yeast the aerobic oxidations of hexosephosphate in presence of pyocyanine are strongly inhibited by iodoacetate. Pyocyanine which is without any influence on the acid formation in the broken up eggs favors the formation of phosphoglyceric acid from hexosediphosphate in suspensions of dry yeast according to observations carried out by Lennerstrand and the writer.

---

[3] In these figures the retention has been accounted for.

The activity of the proteolytic enzyme cathepsin is inhibited by iodoacetate (cf. Bersin, 1935). A proteolytic process induced by this enzyme is excluded as a source of the acid formation in our system.[4] It is to be remembered that iodoacetate does not inhibit the activation process although it could be ascertained that the substance had entered the eggs (Runnström, 1935).

Further evidence of the analogy between the processes induced by freezing and thawing on one side and activation on the other will be presented by Dr. Mirsky. We both find a really significant resemblance between the two processes very probable. The activation " opens doors " (Needham and Needham, 1927) in the interior of the egg. The freezing and thawing breaks down walls allowing mixture of substances separated in the intact unfertilized egg.

I wish to express my sincere thanks to Dr. L. Michaelis for his generous help and valuable criticism and suggestions. I am also very indebted to Dr. A. Mirsky for valuable suggestions and stimulating discussions.

SUMMARY

In previous work it has been found that the activation process is accompanied by an acid formation which has been demonstrated by manometric methods. A comparatively strong acid formation takes place also when the eggs are broken up by distilled water. It seems possible in view of certain observations reported above that the acid formation in both cases has the same chemical basis. The work reported has to be regarded as a first approach to a closer study of this

---

[4] One objection could be raised against these conclusions. The reaction of iodoacetate or fluoride may be too slow to prevent the acid formation in the thawed eggs. The substances in question were added to the egg suspensions before freezing and the concentrations chosen were considerably higher than those generally used. Iodoacetate in the concentration 0.00054 m inhibits fermentation according to Lundsgaard (1930). Lennerstrand and the writer (unpublished data) found 0.003 m iodoacetate—a concentration ten times lower than that used above—to give an inhibition in suspensions of dry yeast already during the first ten minutes following addition. The inhibition was complete, however, only from the second ten-minute period.

question, which has a bearing on the understanding of the activation process.

The eggs have been broken up in the experiments reported by freezing and thawing. During the thawing a considerable acid formation takes place by far exceeding that observed in the activation process. The acid formation was estimated by manometric determinations of the decrease of combined carbonic acid. In a number of experiments the solid particles of the thawed suspensions were centrifuged down. The fluid part has a lower content of combined carbonic acid than the suspension as a whole. Possibly the proteins of the solid particles combine with carbonic acid under the given conditions. The fluid part of the egg suspensions exercises a visible effect on the jelly surrounding the eggs.

Monoiodoacetate 0.03 m, fluoride 0.06 m, and copper 0.0004 per cent have no inhibiting effect on the acid formation. Addition of hexosemonophosphate does not seem to enhance the acid formation. Iodoacetate in presence of hexosemonophosphate does not inhibit the acid formation. Hence it seems extremely unlikely that the acid formation has anything to do with lactic acid formation. Phosphorylation of hexoses or dephosphorylation of phosphoglyceric acid seem to be excluded by the iodoacetate, and the fluoride experiments. Proteolysis under the action of cathepsin is also excluded by the iodoacetate experiments. An oxidation-reduction system like pyocyanine has no influence on the amount of acid formed.

Suspensions of thawed eggs + hexosemonophosphate + pyocyanine form a system with a fairly high oxygen uptake. This is decreased by 30 per cent on addition of 0.03 m iodoacetate or 0.06 m fluoride. This proves that the two substances in question interfere with the carbohydrate metabolism in this system.

LITERATURE

Bersin, Th., 1935. *Ergebnisse d. Enzymforschung,* **4**: 68 (review).
Embden, G., and H. J. Deuticke, 1934. *Zeitschr. f. physiol. Chem.,* **230**: 50.
Harvey, E. B., 1930. *Biol. Bull.,* **58**: 288.
Just, E. E., 1922. *Biol. Bull.,* **43**: 384.
Lundsgaard, E., 1930. *Biochem. Zeitschr.,* **220**: 1.
Needham, J., and D. M. Needham, 1927. *Protoplasma,* **1**: 255.
Örström, A., 1935. *Arkiv f. zoologi,* Stockholm, in press.
Runnström, J., 1930. *Arkiv f. zoologi (Stockholm),* **21**(B): No. 8.
Runnström, J., 1933. *Biochem. Zeitschr.,* **258**: 257.
Runnström, J., 1935. *Biol. Bull.,* **68**: 327.

INFLUENCE OF IODOACETATE ON ACTIVATION AND DEVELOPMENT OF THE EGGS OF ARBACIA PUNCTULATA

JOHN RUNNSTRÖM[1]

(From the Institute of Experimental Zoölogy, Stockholm, Sweden, and the Marine Biological Laboratory, Woods Hole)

Tyler and Schultz (1932) first tried the effect of iodoacetate on fertilization. Their material was the eggs of the worm *Urechis caupo*. They did not find any inhibition of fertilization by 0.02 m solution of sodium iodoacetate.

Runnström (1933*a*) combined 0.002 m sodium iodoacetate with a strong hypertonic solution. The addition of iodoacetate did not change the acid formation following the treatment of the unfertilized *Paracentrotus* egg with the hypertonic solution. Later E. Ellis (1933) tried the influence of iodoacetate on the eggs of *Urechis* and in some few experiments on *Strongylocentrotus purpuratus*. Ellis claims that the iodoacetate is without any influence on the fertilization and division of the egg. The concentrations of the iodoacetate used varied from 0.001–0.01 m. In the fertilization experiments the eggs were placed for five minutes in the solution and then inseminated. In these experiments, as in those by Tyler and Schultz, the question is whether the iodoacetate had time to penetrate the unfertilized eggs before insemination. Ellis proved that iodoacetate penetrates the *fertilized* eggs after an exposure for 95 minutes to an 0.01 m solution of iodoacetate. The glutathione content is estimated in the exposed eggs and found to be only half of the quantity found in the control. One can not take for granted from these figures that the iodoacetate penetrates the unfertilized eggs in five minutes.

R. S. Lillie (1931) mentions that iodoacetate in the concentration of 0.02 per cent (about 0.001 m) is without influence on the heat activation of starfish eggs. Lillie considers the possibility that the iodoacetate does not penetrate under these conditions.

In the present experiments a comparatively high concentration of the iodoacetate (0.03 m) was generally used. The eggs were treated for several (2–6) hours with the iodoacetate containing sea water: during the treatment the eggs were gently shaken to facilitate the diffusion processes. In spite of this the eggs could be fertilized in the iodoacetate sea water. This happened even if the pH of iodoacetate + sea water had been lowered considerably (from 8.1 to 6). In this medium the iodoacetate will probably penetrate more rapidly because of the formation of undissociated molecules of iodoacetic acid. The cytolysis is indeed very strong after two hours treatment but the few eggs still remaining intact can be fertilized after transfer to normal sea water. A normal membrane formation ensues. The microscopic examination revealed in many cases that the eggs although still fertilizable were beginning to present marked structural changes such as precede cytolysis. Eggs in sea water at pH 6 without iodoacetate do not cytolyze. Eggs exposed to iodoacetate for 2–6 hours were subsequently transferred to normal sea water and fertilized. The larvæ developing from these eggs have *shown definite morphological defects.* This proves that the iodoacetate penetrates the unfertilized egg.

---

[1] Fellow of the Rockefeller Foundation, 1933–1934.

The evidence submitted above shows that none of the enzymatic processes inhibited by iodoacetate are necessary for the activation of the egg. Among these glycolysis is especially important; also the proteolytic enzyme cathepsin is inhibited by iodoacetate, as well as all enzymatic or other activity in which the presence of free SH-groups is necessary (cf. the discussion in the recent papers by Michaelis and Schubert, 1934, and Hellerman and Perkins, 1934).

It may be mentioned that the sperm-agglutinating substance secreted by the egg is unaffected by iodoacetate. A series of dilutions was made according to the method of Frank Lillie (1914). No difference in the behaviour of the sperm towards the egg water in presence and absence of iodoacetate could be observed. SH-groups are therefore not involved in the activity of the sperm-agglutinating substance.

The eggs inseminated in iodoacetate show a normal or almost normal increase of respiration upon fertilization. But soon a decrease of the respiration ensues. The presence of 0.002 m sodium pyruvate removes almost completely the inhibition of the respiration by iodoacetate in fertilized eggs. In one experiment, July 23, 1934, the oxygen consumption was measured by the Warburg manometric method, using (1) a control, (2) a sample with addition of 0.03 m iodoacetate, and finally (3) a sample with addition of 0.03 m iodoacetate and 0.02 m sodium pyruvate. The suspension was 6.5 volumes per cent. Each vessel contained 3 cc. egg suspension.[2] The oxygen consumption was, in the three vessels 1, 2, 3, respectively: 133, 88, 122 cu. mm. The average residual respiration in the iodoacetate was 66 per cent. During the first 30 minutes following fertilization the residual respiration was 81 per cent and during the last 30 minutes of the measurement the residual respiration had decreased to 45 per cent. In the mixture iodoacetate + pyruvate the decrease is very small and is due indeed to an inhibition, which was appreciable only during the period 70–100 minutes after fertilization. The development is also blocked by 0.03 m iodoacetate. In the quoted experiment a retardation of the development in presence of iodoacetate was very pronounced. Segmentation took place, but the cells died after some hours even if transferred to normal sea water. The hyaline plasma layer is affected by the iodoacetate. The cells are detached from each other. In some experiments the development was arrested by iodoacetate in a still earlier stage. In one experiment, July 20, 1934, 95 per cent of the eggs remained in the early prophase of the first division as after exposure to n/5,000–n/10,000 HCN. On the living material this stage can be identified by the formation of the "clear streak" close to the nucleus, the contour of which is clearly visible (cf. Fry and Parks, 1934, Fig. 6). In the experiment of July 20, 1934, the inhibition of the development by iodoacetate was thus stronger than in the experiment of July 23, 1934, referred to above. It may not be a mere coincidence that the respiration is also more strongly inhibited in the experiment of July 20, 1934. The oxygen consumption was, during 80 minutes, 124 cu. mm. in the control, and only 50 cu. mm. in presence of 0.03 m iodoacetate. The average residual respiration is 40 per cent. During the first 10 minutes following fertilization the residual respiration is more than 80 per cent, i.e. the initial increase of the respiration is almost normal. In this experiment 0.02 m sodium lactate was added, and again the respiration was restored to a value not far below that of the control 114 cu. mm., i.e. residual respiration 92 per cent. The suspension in this experiment was 7.2 volumes per cent. The number of eggs arrested on the stage of the first prophase in the mixture of sea water, iodoacetate, and lactate was 87 per cent, a very slight improvement of the development indeed (cf. above the value for the iodoacetate without lactate). In no case did a development to swimming larvæ take place in the 0.03 m iodoacetate solutions in sea water.

---

[2] The measurements were begun at the time of fertilization of the eggs and continued for 100 minutes.

The experiments reported show that iodoacetate affects the carbohydrate metabolism in the sea urchin egg in the same way as in other cells. A part of the respiration may be due to an oxidation of carbohydrate breakdown products. When the formation of these is inhibited the respiration may decrease. But it is evident from the experiments with fertilized eggs that iodoacetate interferes also with processes in the cell other than glycolysis as a normal development is not established by the addition of pyruvate or lactate. The addition of lactate or pyruvate, although restoring the respiration, does not save the eggs from the injurious effect of iodoacetate.

Fertilized eggs are decidedly more sensitive to iodoacetate than unfertilized eggs. This may be due to a considerable increase of permeability of the egg to iodoacetate upon fertilization, or it may be due to an increased reactivity of the egg cytoplasm with iodoacetate upon fertilization, or to a combination of these two changes.

As mentioned above, the larvæ developing from eggs treated with 0.03 m iodoacetate *before* fertilization present definite morphological defects. The stomodæum and the oral arms are poorly developed, sometimes lacking, while the organisation and size of the pluteus is in all other respects normal. Addition of 0.02 m sodium pyruvate to the sea water in which the larvæ develop removes almost completely the effect of the treatment by iodoacetate applied before fertilization. Hence it seems probable that a carbohydrate breakdown is a necessary condition in the morphological differentiation of the anterior or " animal " part of the larva. This conclusion is in good agreement with the results obtained by Lindahl (1934, 1935). This author thinks it probable that lithium interferes with some part of the carbohydrate metabolism of the egg. As is well known, lithium salts added to the sea water even in fairly low concentration (e.g. 0.1 per cent LiCl) has a strongly inhibitory influence on the morphological differentiation of the animal region of the larva. A certain decrease of respiration by exposure of the developing eggs to a mixture of 95 per cent carbon monoxide and 5 per cent oxygen also impairs the morphological differentiation of the animal part (Runnström, 1928, 1933*b*). Thus the carbohydrate metabolism must take place under strictly aerobic conditions to warrant a normal morphological development of the animal region of the larva.

This work was carried out during the summer of 1934 at the Marine Biological Laboratory, Woods Hole. My thanks are due to Dr. L. Michaelis for his generous help and valuable suggestions.

SUMMARY

1. It has been proved by the experiments briefly reported above that monoiodoacetate does not inhibit the fertilization process in the *Arbacia* egg. Thus enzymatic or other activities in which SH-groups are involved do not play an essential rôle in the fertilization process. The activity of the sperm-agglutinating substance secreted by the egg is not impaired by iodoacetate. The penetration of iodoacetate into the unfertilized egg is ascertained by the presence of morphological defects in

larvæ reared from eggs removed from the iodoacetate sea water and fertilized in normal medium.

2. Iodoacetate in the concentration (0.03 m) used in these experiments is harmful to the development of fertilized eggs. There is a remarkable difference in resistance between unfertilized and fertilized eggs towards iodoacetate. The normal increase of respiration following fertilization takes place also in 0.03 m iodoacetate but there is soon a considerable decline of respiration, which can be prevented by addition of pyruvate or lactate. A carbohydrate metabolism must be involved in the oxidation processes in the fertilized eggs. The addition of pyruvate or lactate, although restoring respiration, does not inhibit the injury caused to the fertilized egg by the addition of iodoacetate.

3. The morphological defects caused by a treatment of the eggs by iodoacetate before fertilization are prevented by addition of pyruvate to the fertilized eggs. These results have some bearing on the development mechanics of the sea urchin. A carbohydrate breakdown seems to be a necessary condition for the full morphological differentiation of the anterior or animal region of the larva.

LITERATURE

Ellis, E., 1933. *Jour. Cell. and Compar. Physiol.*, **4**: 127.
Fry, H., and M. E. Parks, 1934. *Protoplasma*, **21**: 473.
Hellerman, L., and M. E. Perkins, 1934. *Jour. Biol. Chem.*, **107**: 241.
Lillie, R. S., 1931. *Biol. Bull.*, **60**: 288.
Lindahl, P. E., 1934. *Naturwiss.*, **22**: 105.
Lindahl, P. E., 1936. *Acta Zoologica.* In press.
Michaelis, L., and M. P. Schubert, 1934. *Jour. Biol. Chem.*, **106**: 331.
Runnström, J., 1928. *Acta Zoologica*, **9**: 365.
Runnström, J., 1933*a*. *Biochem. Zeitschr.*, **258**: 257.
Runnström, J., 1933*b*. *Arch. f. Entw. mech.*, **129**: 442.
Tyler, A., and J. Schultz, 1932. *Jour. Exper. Zool.*, **63**: 509.

DIFFERENTIAL OXYGEN UPTAKE OF REGIONS OF LIMULUS OPTIC NERVE AS RELATED TO DISTANCE FROM THE SENSE ORGAN

RITA GUTTMAN

(From the Marine Biological Laboratory, Woods Hole, Mass., and the Zoölogical Laboratory, Columbia University)

Differences in manifestation of physiological activity along the length of a nerve have been the subject of considerable study. In 1933, Marshall and Gerard found that in the most rapidly conducting fibres of the bullfrog sciatic nerve, the velocity of conduction is 27 per cent less in the distal fourth than in the proximal fourth. They found that in the green frog sciatic nerve the rate increases slightly from the proximal to the middle portion and then decreases peripherally. It was thought that a study of respiratory rate along the length of a nerve might also be of interest. The problem is relevant when considered in relation to the question of the nature of the nerve impulse. If the nature of the nerve impulse is not purely physical and is a wave of chemical change, then it is conceivable that metabolic activity may influence the velocity with which the impulse sweeps down a fibre.

The nerve selected for study was the *Limulus* optic nerve rather than the frog sciatic, since for reasons to be elucidated later the former is somewhat better material for differential respiration studies.

In a study of the carbon-dioxide production of the lateral line nerve of the dogfish, Parker in 1928 concluded that no gradient existed in that nerve since the two halves differed but slightly and then not consistently in regard to direction of difference.

Gerard in 1927 investigated the frog sciatic nerve and could demonstrate no respiratory gradient there. On the other hand, Tashiro and Adams (1914), working with an optical method of determination of barium carbonate precipitation for measurement of carbon-dioxide production, claimed that a respiratory gradient existed in the optic nerve of the king-crab, *Limulus polyphemus*. Child (1921) cites the work of Tashiro and adds that differences in rate of metabolism in nerves play a very important part in determining electrical gradients.

Inasmuch as the results of Tashiro and Adams on carbon-dioxide production are high compared with later work and inasmuch as they divided the nerve into but two parts in studying the possibility of the existence of a gradient, it was decided to investigate in detail the oxygen uptake of regions of *Limulus* optic nerve with the Gerard-Hartline microrespirometer (1934) (an apparatus with which the respiration of as small an amount of tissue as 1 mg. may be observed directly from minute to minute) and to divide the nerve into five parts for study instead of only two. The present paper is a report on thirteen experiments.

The optic nerve of *Limulus polyphemus* is one of the longest and most uniform in diameter among the invertebrates, and its fibres are non-medullated. It varies between about eight and twelve centimeters in length, and is protected along its entire course by a uniform non-nervous sheath, which was not stripped from the nerve in these experiments. Male *Limuli* were used since the dissection is easier there. The nerve was obtained intact from the sense organ to the brain in most cases. Dissection was always begun at the eye and proceeded centrally. Every effort was made to avoid undue manipulation.

The nerve was cut into five approximately equal segments and each piece inserted by means of a fine hooked glass needle into a calibrated glass capillary. One end of the capillary was sealed with vaseline and at the other end a drop of half molar sodium hydroxide was inserted. The capillary was then placed in a sealed glass chamber of much larger volume and the whole immersed in a constant temperature water bath. Movement of the drop was observed with the aid of a horizontal microscope equipped with a filar-ocular micrometer.

Carbon-dioxide evolved was absorbed by the alkali, which also acted as an index drop, and since the ratio of the volume of the outer glass tube to the capillary volume was more than 5000 to 1, the rate of movement of the drop gave a direct measurement of the oxygen uptake.

Instead of studying the respiration of cut sections of the nerve, it would have been more desirable to study the several regions in the intact excised nerve, or better still, *in situ* in the living animal. Since the technique for this has not been perfected, the next best procedure was adopted.

The typical respiration curve of a nerve or portion of a nerve is plotted in Fig. 1. Owing to the recent handling and dissection, respiration is high at first, then drops to a uniform steady rate which is maintained for about five or six hours, falls again when the oxygen present is insufficient to support maximal respiration, and then vanishes. The respiration studied in these experiments is the uniform steady rate.

In discussing his results with Dr. Tashiro, I discovered that the aspect of gas exchange he was studying was the high activity represented in the first part of the curve, since he measured carbon-dioxide production immediately after dissecting out the nerve and for a short period of time —usually not more than fifteen minutes. This may explain the differences in his results and the results obtained in these experiments as regards both relative and absolute rates. In regard to relative rates, different amounts of time are required to achieve a steady state in different nerves and regions of nerves. With respect to absolute rates, results would obviously be higher in his experiments if the first part of the curve only were utilized.

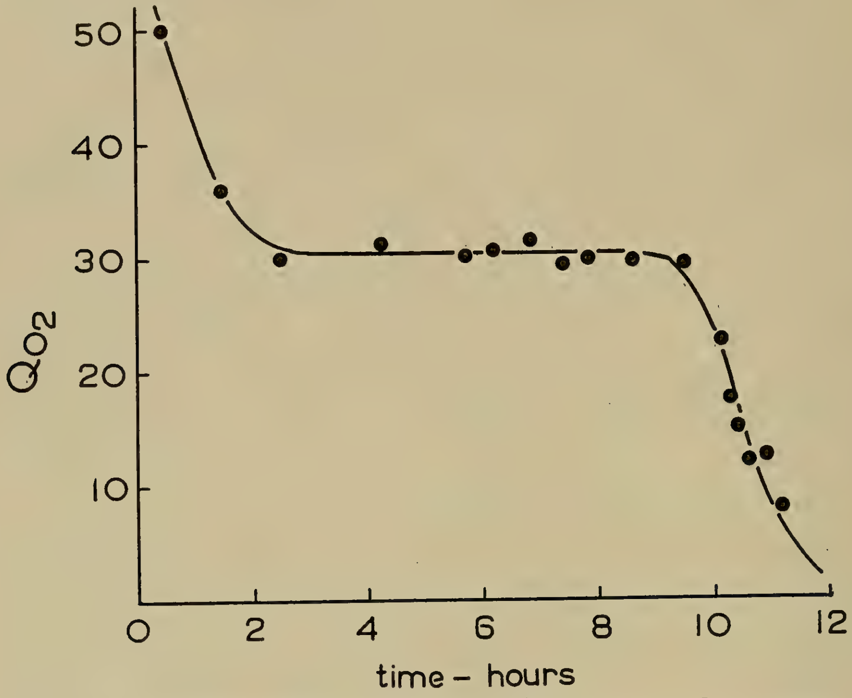


FIG. 1. Typical oxygen uptake of a nerve or portion of a nerve. Q_{O_2} represents cu. mm. oxygen per gram moist weight per hour.

That the high portion is not an artefact entirely due to movement of the drop in reaching an equilibrium was shown by control experiments where sodium hydroxide was inserted into a capillary containing no tissue.

The steady rates of the five portions of a nerve were compared. The results are summarized in Table I. "*A*" represents the fifth of the nerve most proximal to the brain; "*E*", the fifth of the nerve most distal to the brain, and "*B*", "*C*" and "*D*", intermediate regions. In every case where the most proximal fifth is present (in two early cases, Experiments 11 and 13, the nerve was not dissected out entirely to the brain) the proximal fifth has a higher respiratory rate than the distal one. In every case except the last two, maximal respiration occurs between the two ends of the nerve. The average difference between maximum and minimum is 36 per cent, which is far beyond the precision of the instrument.

Both right and left nerves were studied. In Experiments 1, 2 and 3, 4, which represent paired nerves, no striking differences were observed between right and left nerves. Respiration of the forebrain was studied in two cases and found to be higher than in any part of the nerve.

Tashiro and Adams (loc. cit.) based their belief in the existence of a gradient in the optic nerve of *Limulus* on observations that the respiration of the distal half was higher than the respiration of the proximal half. Yet if we compare the distal two-fifths with the proximal two-fifths in these measurements, we find the reverse situation to be true, i.e. not the distal but the proximal two-fifths is found to have the higher respiratory rate in each case. It was partly on the assumption that respiration of a nerve is highest at the point of stimulation, i.e. the sense organ, that Tashiro based his theory of nerve conduction outlined in "A Chemical Sign of Life" (1917).

The effect of temperature upon the differential respiratory rate was also investigated. The relationships found above remained unaltered throughout the temperature range: 16 to 31° C.

Q_{O_2} of regions of Limulus optic nerve

Expt.	Temp. (°C.)	Brain				Eye	Prox. 2/5	Dist. 2/5
		A	B	C	D	E		
1	31.0	99.7	109	118	95.0	82.5	105	89.0
2	31.0	102	92.0	117	101	81.0	98	91
3	28.3	65.1	62.5	76.1	53.4	53.3	63.7	53.3
4	28.3	68.1	67.6	76.1	55.6	47.3	67.9	51.5
5	19.3	36.4	49.9	40.6	43.4	34.6	43.1	39.0
6	18.0	28.5	32.9	34.4	25.1	23.7	30.8	24.5
7	17.1	30.0	38.6	30.6	27.8	25.2	34.4	26.6
8	17.1	40.1	36.3	?	29.8	32.4	38.2	31.1
9	16.1	15.3		17.7	16.5	9.4	15.3	13.0
10	16.1	32.2	32.3	23.2	27.8	15.2	32.3	21.6
11	16.1	—	14.7	19.8	19.0	12.9	—	—
12	16.1	32.4	30.7	29.5	31.1	20.9	31.5	26.0
13	16.1	—	23.6	18.1	21.2	31.6	—	—

A is most proximal fifth. *E* is most distal fifth.

Q_{O_2} represents cu. mm. oxygen at 0° C. per gram of tissue (moist weight) per hour.

SUMMARY

The differential rate of oxygen uptake of the optic nerve of *Limulus polyphemus* was investigated by means of a differential volumeter. Thirteen experiments are reported. It was found that:

1. In every case the respiration of the distal end was lower than that of the proximal region. The respiration of the distal end was lower than that of any other region of the nerve in all the cases studied but one.

2. A maximal rate was found in ten out of twelve cases along the central portion of the nerve. Thus no gradient in the accepted sense of the word of oxygen uptake exists in the nerve.

3. The average difference between maximal and minimal rates in a nerve was 36 per cent.

LITERATURE CITED

Child, C. M., 1921. The Origin and Development of the Nervous System. University of Chicago Press, Chicago.

Gerard, R. W., 1927. Studies on nerve metabolism. II. Respiration in oxygen and nitrogen. *Am. Jour. Physiol.,* **82**: 381.

Gerard, R. W., and H. K. Hartline, 1934. Respiration due to natural nerve impulses. A method for measuring respiration. *Jour. Cell. and Comp. Physiol.,* **4**: 141.

Marshall, W. H., and R. W. Gerard, 1933. Nerve impulse velocity and fiber diameter. *Am. Jour. Physiol.,* **104**: 586.

Parker, G. H., 1928. Carbon dioxide from the nerves of cold-blooded vertebrates. *Am. Jour. Physiol.,* **86**: 490.

Tashiro, S., 1917. A Chemical Sign of Life. University of Chicago Press, Chicago.

Tashiro, S., and H. S. Adams, 1914. Comparison of the carbon-dioxide output of nerve fibers and ganglia in Limulus. *Jour. Biol. Chem.,* **18**: 329.

CHANGE IN RATE OF RESPIRATORY METABOLISM IN A TELEOST FISH INDUCED BY ACCLIMATIZATION TO HIGH AND LOW TEMPERATURE

NELSON A. WELLS

(From the Scripps Institution of Oceanography, University of California, La Jolla, California)

Introduction

The possibility that the rate of oxygen metabolism of fishes at any given temperature may be conditioned by the temperature of the water from which the animals are taken seems not to have been considered in the work so far published on oxygen metabolism of fishes. The probability that a change in the rate of metabolism of cold-blooded vertebrates would be effected by acclimatization of the animals to different temperatures has been expressed by Montuori (1913), Krogh (1916), Baudin (1932), and Benedict (1932). Krogh reasons that since fishes are found living normally over such a wide temperature range, there must be some adjustment of the metabolic processes to temperature, that " animals living at a very low temperature should show a relatively high standard metabolism at that temperature compared with others living normally at a high temperature."

Benedict finds from his study on the metabolism of reptiles that the warmer the environment from which the animals are procured, the lower is their standard metabolism. He states, however, that " the effect of geographical location of the animal cannot be considered to be clearly demonstrated, since the size of the various species is shown to play a considerable rôle."

In another publication (Wells, 1935*b*) I have discussed the change in the metabolic rate of *Fundulus parvipinnis* after these fishes had been subjected to high and low temperatures for definite intervals of time. This paper is based upon a continuation of that work, using another species of fish and employing greater temperature differences between the cold and warm-adapted fishes.

Material and Methods

The fishes used in these experiments were *Gillichthys mirabilis*, known to fishermen as " mud suckers," and used extensively as bait because of their extreme hardiness. They will live equally well in fresh or salt water and will remain alive for hours out of water if they are kept in a moist environment. Ordinarily they may be kept indefinitely under laboratory conditions since they are not subject to the disease which is so fatal to *Fundulus* (Wells and ZoBell, 1934). They remain healthy and normal over a wide temperature range (from 8° to 35°).[1]

The water for the warm aquaria was heated by passing it through a block tin coil sealed into an ordinary hot water tank. It was found necessary to remove the excess of dissolved gases from the sea water after it had been heated, because at the high water temperatures the supersaturated sea water caused the well-known " gas bubble disease " of fishes. The excess gases were removed by passing the water in a very thin layer over a cascade constructed from two ordinary glass washboards. Since the water temperature was approximately three degrees higher at the top of the cascade than it was when it reached the tank, the method was quite effective and resulted in maintaining the water in the stock tanks slightly below the saturation point at any desired temperature.

The cold water was obtained by passing the sea water through a block tin coil immersed in a brine tank, cooled with an electric refrigeration unit. With heating and cooling devices it was possible to maintain running sea water at temperatures from 8° to 40°.

Elsewhere (Wells, 1935*a*) the apparatus used for the determination of the oxygen metabolism of fishes has been described in detail. Briefly, it provides a continuous flow, constant temperature system, in which the rate of flow can be maintained at any desired value, and the temperature can be controlled to within 0.1° C. Oxygen was determined by the well-known Winkler method.

EXPERIMENTAL PROCEDURE

In December, 1934, two groups of 9 fishes each,[2] of as nearly equal mean size as it was possible to select, were placed in respiratory chambers. The first group, to be designated A_1, was taken from a tank in which the running sea water had been maintained between 32.8° and 34.5°, but usually at 33°, for a period of 17 days. Group A_2 had been kept for the same interval of time in cold water the temperature of which varied from 10.5° to 13°, but during most of the time the temperature was *ca.* 11°. Both groups were placed in respiratory chambers (see Wells, 1935*a*, p. 205) and the chambers immersed in the constant temperature bath at a temperature of 22°. The first value of their oxygen consumption was taken 30 minutes later. Other values were obtained during the daytime for a period of 50 hours. These values are plotted in Fig. 1, *A*.

---

[1] All temperatures are expressed in ° C.

[2] The average weight of the *A* group was 8 grams and of the *B* group, 8.9 grams.

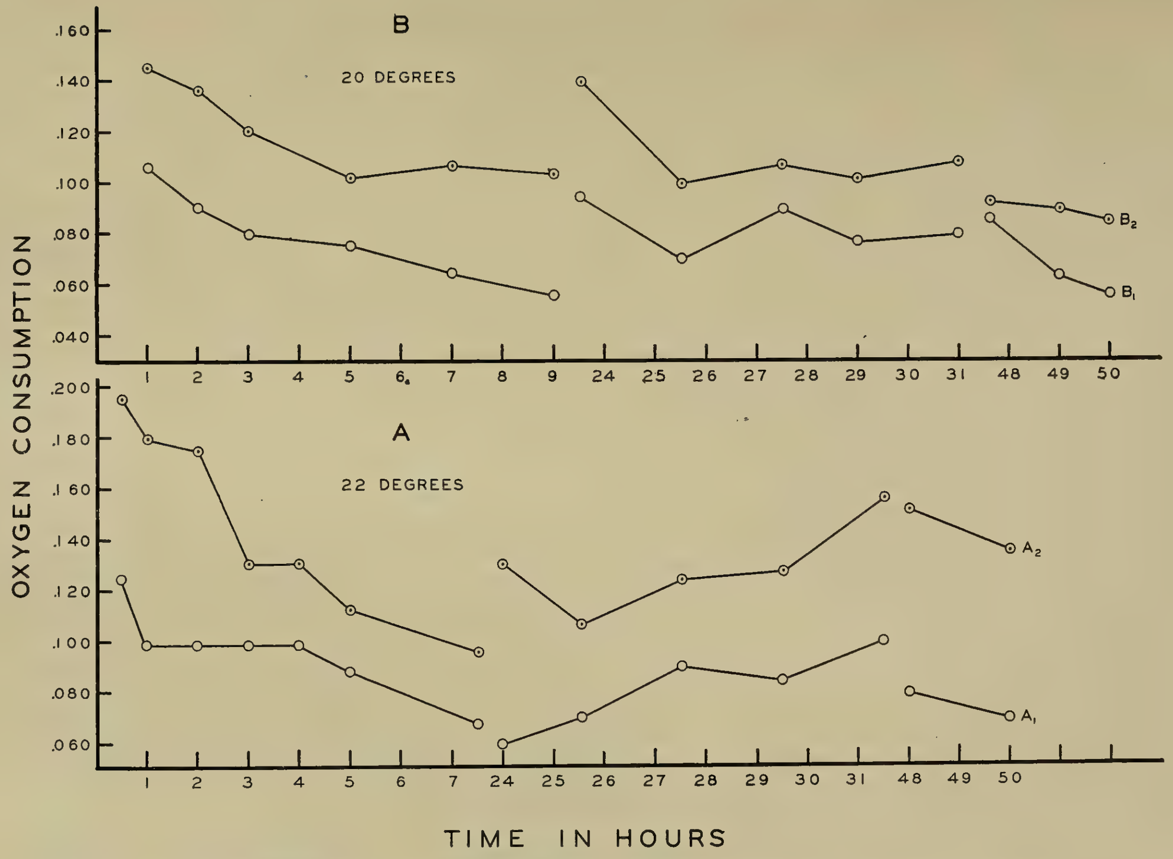


FIG. 1. Oxygen consumption at a common temperature of fishes acclimatized to different temperatures. The ordinates represent the oxygen uptake of the fishes in grams per hour at a temperature of 22° in *A* and 20° in *B*. The abscissas represent time in hours from the moment of transfer of fishes to the respiratory chambers. The upper curves in both *A* and *B* are the values obtained from the fishes of cold water history, and the lower curves, the values from the ones of warm water history.

On January 3, two more sets of fishes (Experiment 2), 16 in each set, were selected. The warm-adapted fishes, group B_1, had been kept in running sea water the temperature of which varied from 29.6 to 31.6 for a period of 20 days, and group B_2, the cold-adapted fishes, had been maintained at a temperature level between 10° and 12° for the same interval of time. In this experiment, since the temperature of the acclimatization period of the " warm " fishes had been 2½ degrees lower, the metabolism values were obtained at 20°. The first value was secured one hour after introduction into the respiratory chambers and, as in the first experiment, values of the oxygen consumption were obtained throughout the daytime for 50 hours. These values are plotted in Fig. 1, *B*.

In both of these experiments a standard feeding schedule was adopted. The fishes in each group were fed all the live food they would take during the period of acclimatization and were deprived of food for an interval of 24 hours prior to being placed in the respiratory chambers.

Discussion and Results

There is undoubtedly a change in the " normal metabolism " (Wells, 1932) of fishes effected by acclimatizing them to high and low temperatures. An excellent visual indication of this is observed when comparing the opercular breathing rhythm of two groups of fishes from cold and warm water at a common intermediate temperature. Fishes with a cold water history, even after 10 days at the intermediate water temperature, breathe at nearly twice the rate of that of the warm history fishes. A detailed discussion of the change in opercular breathing rhythm and the resistance to anæsthetics, asphyxiation, and cyanide poisoning produced by acclimatizing fishes to high and low temperature, will be found elsewhere in this journal (Sumner and Wells, 1935).

In another paper (Wells, 1935*b*), I have presented the data to show that there is not only a change in the rate of respiratory metabolism of *Fundulus parvipinnis* when these animals are acclimatized to high and low temperatures, but that there is a seasonal rhythm even when these fishes are maintained at a constant temperature. The seasonal change, in view of the results presented in this paper, leads one to the conclusion that the fish possesses a buffering mechanism, so to speak, which enables it to adjust itself to the seasonal changes in water temperature, thereby maintaining a practically constant rate of oxygen metabolism. My reason for this statement is, that as the temperature difference to which the fishes are acclimatized increases, the oxygen consumption, at a common temperature, of the cold-adapted fishes increases over that of the " warm " fishes. In my experiments on *Fundulus*, where I was only able to maintain a temperature difference of approximately 15°, the metabolism of the cold fishes was 20.4 per cent higher at a temperature of 12° than the warm fishes. In the experiments presented in this paper, however, where the acclimatization temperatures were farther apart, the difference between the two groups at a common temperature is greater. In the first experiment (Fig. 1, *A*) the acclimatization temperature difference was, on an average, 22° and the average increase of the cold fishes over the warm ones, after 24 hours in the respiratory chamber, was 67 per cent at 22°. In the second experiment (Fig. 1, *B*), when the acclimatization temperature difference was approximately 19.5°, the increase in oxygen consumption of the cold fishes at a common temperature of 20° was only 37 per cent.

Now let us consider the data presented in Fig. 1. The ordinates represent the oxygen uptake of the fishes in grams per hour at a temperature of 22° in *A* and 20° in *B*. The abscissas represent time in hours from the moment of transfer of the fishes to the respiratory chambers. The upper curves in both *A* and *B* are the values obtained from the fishes of cold water history and the lower curves are values obtained from the ones of warm water history. It is obvious from an inspection of these two sets of curves (Fig. 1) that the oxygen metabolism of the warm-adapted fishes is lower than that of the cold-adapted ones when determined at the intermediate common temperatures. In every case the simultaneously determined values of the warm-adapted fishes fall below those of the " cold " fishes. With few exceptions, the trend of each curve in a pair is the same.

In the *B* series curves, where the values were obtained at 20° and the warm fishes had been maintained at a 2.5° lower acclimatization temperature than in the *A* series, there is an indication that the induced difference of metabolic rate of the two temperature groups is disappearing. In the *A* groups, on the other hand, the values on the second day are farther apart than on the first, and still farther on the third day. The increase on the third day of the cold fishes over the warm ones was 98 per cent. This difference was substantiated in the differential reaction of these two groups to urethane after the fishes were removed from the respiratory chambers (Sumner and Wells, 1935). It must not be overlooked that these data are too meager to justify concluding that the 2.5° acclimatization temperature difference is entirely responsible for the difference in reaction shown in these two experiments. The difference between the reaction of the two sets, *A* and *B*, may be due, in part, to the different temperatures at which the values of the respiratory metabolism were determined. I have shown elsewhere (Wells, 1935*a*) that the relation of oxygen consumption to temperature, in fishes, does not follow a smooth curve, but may increase markedly at certain temperatures; and, further, that the temperatures at which these increases occur may be conditioned by the temperature of the water from which the fishes are taken, that is to say, the temperature to which the animals have been acclimatized. It is obvious from a comparison of the two graphs *A* and *B* that the rate of oxygen consumption was less stable or constant at 22° than it was at 20°. This is indicated by the greater variability of the consecutive values of both the cold and warm water fishes.

However, a point that seems to me to have significance is the fact that the warm-adapted fishes, acclimatized to a 2.5° higher temperature in the *A* series than in the *B* series, had practically the same rate of metabolism at 22° as the *B* series had at 20° (.079 and .077 respectively)[3] while the cold-adapted fishes in the *A* series were 28 per cent higher at 22° than the cold-adapted *B* series at 20°. If, as I have postulated, these fishes are able to adjust their rate of metabolism to temperature changes, these results are predictable. The *A* and *B* series warm fishes have been subjected in both cases to an effective drop in temperature of approximately 11°; therefore, if their rates of metabolism at the two high temperatures had been established at essentially the same level, the 11° drop should affect the two groups of fishes equally. Both sets of cold fishes, on the other hand, had been acclimatized to the same temperature, and the effective increase in temperature was two degrees higher for the *A* series than for the *B* series, and the metabolism of the *A* group at 22° should be higher than the metabolism of the *B* group at 20°.

The data presented in this paper certainly warrant considering that at least two species of fishes are able to achieve a certain amount of adjustment of their oxygen metabolism to the temperature changes of their environment. How rapidly and to what extent they can make the adjustment is yet to be determined. We have an indication from the breathing rhythm (Sumner and Wells, 1935) that an adjustment once made persists for several days. The permanency of the adjustment is no doubt a function of the duration of time at which the animals are maintained at the high or low temperatures. These results, in conjunction with the results of Sumner and Wells, on the differential effect of anæsthetics, asphyxiation, and cyanide poisoning on fishes acclimatized to different temperatures, indicate that further work would be highly desirable.

---

[3] The rates of metabolism are obtained by averaging all values secured after the fishes had been in the respiratory chambers for a period of 24 hours (see Wells, 1932).

Summary

The results of two experiments are presented showing the effect on the oxygen metabolism produced by acclimatizing fishes to high and low temperatures. It is concluded from an analysis of these data that fishes acclimatized to a high temperature have a much lower rate of metabolism at a common intermediate temperature than do fishes acclimatized to a low temperature, and that the magnitude of this difference is a function of the difference between the acclimatization temperatures.

LITERATURE CITED

Baudin, Louis, 1932. Respiration du poisson (*Carassius auratus*) anesthésié à la tricaïne et sour mis à une élévation brusque de température. *Compt. rend. Soc. de Biol.,* **110**: 19, 235–237.

Benedict, Francis G., 1932. The physiology of large reptiles. *Carnegie Institution of Washington,* Publ. No. 425.

Krogh, A., 1916. The respiratory exchange of animals and man. Longmans, Green, and Co.

Montouri, A., 1913. Les processes oxidatifs chez animaux marins en rapport avec la loi de superficie. *Arch. Ital. de Biol.,* **59**: 213.

Sumner, F. B., and N. A. Wells, 1935. Some relations between respiratory metabolism in fishes and susceptibility to certain anæsthetics and lethal agents. *Biol. Bull.,* in press.

Wells, N. A., 1932. The importance of the time element in the determination of the respiratory metabolism of fishes. *Proc. Nat. Acad. Sci.,* **18**: 580.

Wells, N. A., 1935*a*. The influence of temperature upon the respiratory metabolism of the Pacific killifish, Fundulus parvipinnis. *Physiol. Zool.,* **8**: 196.

Wells, N. A., 1935*b*. Variations in the respiratory metabolism of the Pacific killifish, Fundulus parvipinnis, due to size, season, and continued constant temperature. *Physiol. Zool.,* **8**: 318.

Wells, Nelson A., and Claude E. ZoBell, 1934. Achromobacter ichthyodermis, n. sp., the etiological agent of an infectious dermatitis of certain marine fishes. *Proc. Nat. Acad. Sci.,* **20**: 123.

SOME RELATIONS BETWEEN RESPIRATORY METABOLISM IN FISHES AND SUSCEPTIBILITY TO CERTAIN ANESTHETICS AND LETHAL AGENTS

F. B. SUMNER AND N. A. WELLS

(*From the Scripps Institution of Oceanography, University of California,*
La Jolla, California)

INTRODUCTION

It has long been known that gradual acclimatization may enable an animal to withstand temperatures so high that they would speedily prove fatal were the change made abruptly. There is some evidence, though of less extent, relating to extreme reductions of temperature. It is a curious fact that tolerance to high temperatures, once acquired, may persist for considerable periods after the return of the organism to the temperature from which it was taken, or even to a considerably lower one. Some rather lasting, though probably not permanent, change has been brought about in the physical or chemical properties of the organism. Regarding the nature of this change, various hypotheses have been offered. Certain authors have suggested the probability of a rise in the coagulation point of proteins, due to a loss of water in the tissues, as a result of exposure to higher temperatures.[1] Others have pointed to the rôle played by differences of metabolic rate in determining the resistance of organisms not only to heat but to other lethal agents.

Experiments by Child (1913 and later) " demonstrate the existence of a relation between the length of life (physiological resistance) of the animals or pieces in KCN, alcohol, and various other anesthetics and the rate of the metabolic reactions or certain of them, probably the oxidations." Miss Behre (1918), working in Child's laboratory, found for a planarian that transfer to a higher temperature increased the metabolic rate and, conversely, that transfer to a lower temperature decreased this. Much more important, for present purposes, was the fact that any such transfer was followed by a regulative process, so that a worm which had been kept for a certain length of time at a given temperature displayed a definitely higher rate of metabolism than one which was transferred to this directly from a higher temperature, and a definitely lower rate than one which was transferred directly from a lower temperature. The indices of metabolic rate employed by her were susceptibility to poisoning by KCN, and the production of CO_2.

---

[1] Davenport and Castle, 1895.

Loeb and Wasteneys (1912), while not concerning themselves with respiratory metabolism, conducted some highly instructive experiments with *Fundulus* (presumably *F. heteroclitus*), a small marine teleost. Their objects were, among other things, " to find out the maximum temperature into which they [fishes kept at 10° to 14° C.] could with impunity be [transferred] suddenly" and " how long it takes to immunize the fish against the harmful effects of a sudden transfer to a temperature of 35° C."

Probably the most striking results from these experiments relate to the high persistence of the immunity obtained, in comparison with the duration of the immunization process. Thus, " the immunity against a temperature of 35° acquired by keeping the fish for two days at 27° is not lost or weakened if the fish are afterwards kept as long as thirty-three days at a temperature of from 10° to 14°." (Experiments not continued longer than this.) More surprising yet, perhaps, " the immunity against a temperature of 35° is also maintained if the fish are kept after the two days' exposure to 27° for two weeks at a temperature of 0.4° C." Moreover, " it is not necessary to expose [fishes] continuously to a high temperature. An intermittent exposure to a higher temperature during a number of hours each day will bring about the same effect." (See, also, Hathaway, 1928.)

Wells (1935, 1935a, 1935b) has measured the consumption of oxygen for another species of *Fundulus* (*F. parvipinnis*) and for one species of goby, under various conditions of temperature and season. He has shown not only that the " metabolic rate," as measured by oxygen consumption, is a function of the temperature at the time of the experiment, but that the rate for any given lot of fishes is greatly influenced by the recent temperature history of the animals in question. Thus, in one experiment with gobies, two lots of nine fishes each, which had been kept for 17 days at approximately 11° and 33.5° C., respectively, were tested after both lots had been held at a common, intermediate temperature for 48 hours. The former lot gave a figure for oxygen consumption almost exactly twice as great as the latter (Wells, 1935b).

The agreement between these results with fishes and those obtained by Miss Behre with planarians is obvious. The suggestion is also obvious that the physiological differences which express themselves as differences in oxygen consumption may be directly related to, if not identical with, those upon which differences in immunity to high temperatures depend. If a fish which has been freshly subjected to a much higher temperature exhibits, at this temperature, a higher rate of respiratory metabolism than a fish which has been acclimatized to this condition, it is not surprising that the former fish should be more likely to succumb than the latter. For it does not seem improbable that one factor, at least, in the lethal effect of high temperatures is a fatal increase in the rate of oxidation.

Loeb and others have attributed to cyanides an effect in reducing the ability of living matter to utilize oxygen, while Child has employed the relative susceptibility of an organism, or part, to potassium cyanide and some other substances as a useful index of its metabolic rate. It therefore seemed to us desirable that fishes having different histories in relation to temperature should be subjected to the influence of certain lethal agents, in order that comparisons might be made among these fishes in respect to their susceptibility.

Although about a hundred experiments of this sort have already been made by us, the present report is to be regarded as a " preliminary " one, even though it may not be possible to continue these studies in the future. Many essential phases of the subject have been left untouched, and certain lines of experimentation have not been brought to a conclusive issue. We have been handicapped, at times, by lack of material, and the control of water temperature was not always adequate. However, no reservations seem necessary as regards our major lines of evidence. In general, we may say that the " horizontal " comparisons, i.e., comparisons of the performance of contrasted lots of fishes at any one time, are of much more importance than " vertical " comparisons, made between corresponding lots of fishes in different experiments conducted at different times. This, because of probable seasonal changes in the fishes; likewise, because of temperature changes in both our " warm " and " cold " tanks, and particularly in the " intermediate " tanks which commonly held untreated sea water.

The temperature of the " cold " aquaria varied somewhat with the season, but lay, nearly always, between 10.5° and 12.0° C., rising occasionally above 13°. The temperature of the " warm " aquaria, in most experiments, lay between 32° and 35°, though it surpassed both of these limits on a few occasions. The duration of subjection to these temperature extremes varied from 24 hours to several months. Except for a brief period, the " intermediate " tanks were supplied with sea water directly drawn from the laboratory supply. This naturally varied with the season, ranging from 15° to 20.5° [2] during the period covered by these experiments.

The fishes used in these experiments were our local killifish, *Fundulus parvipinnis,* and the long-jawed goby, *Gillichthys mirabilis.* Those of both species varied considerably in size, the former ranging from 6

[2] 20°–23° during a few weeks in winter, when it was artificially warmed.

to 8 centimeters in length, the latter from 8 to 15 centimeters. So far as possible, comparisons were restricted to fishes of approximately the same size.

In the greater number of our experiments, our procedure consisted in keeping fishes for periods of varying length in the three tanks just referred to, then returning them to a common temperature and subjecting them to the various anesthetics or lethal agents. In a limited number of the experiments the fishes were tested at the temperatures to which they had become acclimatized. As anesthetics, ethyl urethane (commonly a 1 per cent solution,[3] chloretone (1/20 to 1/10 of saturation), and ether were used; as lethal agents NaCN (1/1,000 M), KCN (1/3,000 M), de-oxygenated sea water, and in one experiment, heat. The time when each fish succumbed to the treatment in question was recorded. A fish was regarded as having "succumbed" when every visible trace of activity had ceased. Since the respiratory movements of the mouth and opercula persisted after other movements had ceased, these were carefully watched. In experiments with cyanide and with de-oxygenated sea water, responsiveness to poking with a glass rod was taken into consideration. With each agent employed, the moment of "succumbing" was commonly fairly well defined, and no serious difficulty was encountered in determining it. "Succumbing," in this sense, was by no means equivalent to death, however, since many of the fishes recovered completely when returned to fresh sea water. Indeed, one of the important data which we recorded in many of our experiments was the recovery or non-recovery of each individual fish when removed from the adverse conditions.

Another important item of observation was the rate (number per minute) of externally visible respiratory movements made by the mouth and opercula. Strongly correlated with the rate of these movements was their depth, differences in which could be observed in most cases, though they could not be quantitatively recorded.

Experimental Results

In the present circumstances, no full record of the various experiments seems called for. The results may be summarized rather briefly. It is hoped that a more detailed report, based upon a more adequate series of experiments, may be presented in the future.

The first experiments to be mentioned are two which related to the susceptibility of fishes to the agents in question, while kept at the temperatures to which they had become acclimatized. In tests with a few specimens each of *Fundulus* and *Gillichthys,* the fishes in the "warm" tank proved to be consistently more susceptible to urethane than those in the "cold" tank. Rather unexpectedly, however, the fishes in the "intermediate" tank showed a higher resistance than either of the other lots, but it must be stated that the temperature of the "intermediate" water was at this time much closer to that of the "cold" than to that of the "warm."

---

[3] This was prepared by mixing one part of a 10 per cent solution of urethane in distilled water with 9 parts of sea water.

These experiments are unfortunately altogether too limited in number. It should be pointed out, however, that the results are completely in accord with the observed respiratory rates of the "warm" and "cold" fishes in their own media. Thus three fishes which had been acclimatized to the "cold" tank (9.7° at time of count) gave a mean rate of 46 respirations per minute, as compared with three "warm" fishes (33°), which gave a mean of 96. The periods of residence in the two tanks had been 120 and 61 days, respectively. In another case, 6 fishes each of "cold" and "warm" history (131 and 72 days), tested in their own tanks (11° ± and 32° ±), gave means of 46 and 89, respectively. Our results as a whole show that susceptibility to urethane and to various other drugs is a function of the metabolic rate, as manifested both by oxygen consumption and by visible respiratory movements.

In by far the greater number of our experiments the fishes, after acclimatization to the various temperatures, were subjected to the anesthetic or lethal agent at a common temperature. For the most part, this common temperature was an intermediate one, usually the air temperature of the room.

In the numerous experiments in which the test was made immediately or shortly after the transfer of the fishes to a common temperature, and in which the animals were subjected to urethane, the results were uniformly consistent in their essential features. With scarcely an exception, fishes of "warm" history outlasted those of "cold" history. In other words, fishes transferred from a higher to a lower temperature resisted the effects of the anesthetic longer (usually much longer) than those transferred from a lower to a higher temperature. For the most part, fishes of "intermediate" temperature history proved to be intermediate in their susceptibility to the anesthetic, though this relation was not as constant as that between "cold" and "warm" specimens.

A number of experiments were performed in which the fishes were kept for varying periods in water of intermediate temperature between their removal from the "cold" and "warm" tanks and the test of susceptibility. The persistence of wide differences in oxygen consumption, 48 hours (±) after the discontinuance of the temperature differences, has been discussed by Wells (1935b). The 18 fishes which were employed for this purpose in his "experiment A" were subjected to urethane at the close of the latter experiment. The "cold" lot succumbed within periods of from 5 to 55 minutes, the mean being 38. Only one of the "warm" lot, on the other hand, had succumbed by the close of 55 minutes, when the experiment was discontinued.

In the foregoing experiment, the fishes had previously been subjected to the temperature extremes for a period of 17 days. In another experiment, the periods of acclimatization were 118 days (cold) and 59 days (warm), and the interval between removal from these temperatures and the test of susceptibility was 14 days. The mean periods required for the anesthesia of the 9 fishes of each lot were 74 minutes ("cold") and 83 minutes ("warm"). This was not a very wide difference, particularly as there was considerable overlap between the periods of resistance of the two lots. A more striking difference was shown in their mean respiratory rates, these being 102 respirations per minute for the "cold" lot and 54 for the "warm." After 28 days at a common temperature, these same fishes[4] showed virtually identical respiratory rates, while their relative susceptibility to urethane had undergone a slight reversal.

The most serious defect in our experimental data is the inadequate basis which they afford for a comparison of the effects of long and short periods of subjection to temperature differences. We have very few data which permit of direct comparisons between fishes having identical history except for the length of this period. A few experiments were, however, undertaken for the purpose of comparing the reactions of fishes which had been acclimatized to warm water for 2 days with those of fishes which had been kept for much longer periods (8 and 35 days) at the high temperature. The latter (comprising both *Fundulus* and *Gillichthys*) showed a somewhat higher resistance to urethane than the former when tested at this same temperature. But the differences were not great and the numbers of individuals were so small that no great stress can be laid upon these results.[5]

On the other hand, it is interesting to note that in four experiments with *Fundulus* in which the period of acclimatization was only 24 hours, the mean figures for the "cold," "intermediate," and "warm" lots (4 of each) were approximately 20, 24, and 76 (+) minutes.[6] Taking these figures at face value, the difference between these "cold" and "warm" lots was even greater for these 24-hour fishes than for many of those which had been acclimatized for much longer periods. And again with gobies, it happens that the highest figure of all (135 +) is that recorded for a fish which had been kept only one day in the warm tank.

---

[4] Except that two of the "cold" lot had succumbed to the first treatment with urethane.

[5] An obvious procedure would be to test the same lot of fishes after varying intervals, and such experiments, indeed, were made. But we found some evidence that resistance to urethane increased with successive trials.

[6] This figure is too low since some of the experiments were discontinued before the "warm" individuals had completely succumbed.

Furthermore, in two experiments with fishes (*Fundulus*) which had undergone acclimatization for only two days, the 5 "warm" fishes displayed a markedly higher resistance than the 5 "cold" ones, even after being kept from 20 hours to 2 days at a common temperature.

Despite the paucity of these last data, therefore, it seems evident that considerable and rather persistent differences in susceptibility to urethane may be brought about in these fishes in one or two days' exposure to the differing temperatures here employed.

Several experiments with chloretone in which gobies (12 fishes) were used, gave results quite in harmony with those from urethane. Large and consistent differences were shown between the fishes of "warm" and "cold" history which had been kept in their respective tanks for 2 to 3 and 4 to 5 months, respectively, and tested immediately after removal at an intermediate temperature.

Experiments with ether were unsatisfactory, since it was not easy to distinguish the point of complete anesthesia. In only one of four experiments was a significant difference noted, this being in the same direction as in the case of the anesthetics previously considered.

Our experiments with cyanides are more nearly comparable with the experiments of some of those who have worked in this field upon invertebrates. It did not, however, seem a foregone conclusion that experiments upon fishes would yield results parallel to ones upon such simple organisms as planarians. The toxicity of CN to a fish might depend upon factors other than its effect upon the utilization of oxygen. Indeed, our first experiments of this sort led us to doubt whether susceptibility to cyanide, in these animals, bore any relation to metabolic rate.

In the first five of these experiments, 0.001 M solutions of NaCN in sea water were used. The fishes (gobies) had been exposed to the differing temperatures for relatively brief periods (3 to 15 days). In four of these experiments, which were conducted at "intermediate" temperatures (20° to 22°), no significant differences were noted in the survival time of the different lots of fishes. In a fifth experiment, however, in which "intermediate" and "warm" fishes were subjected to the cyanide solution at 31° (the temperature of the "warm" water at the time), the mean survival times for the two lots of three fishes each were: "intermediate," 12 minutes; "warm," 33 minutes.

The remaining 7 experiments with cyanide differed from the preceding ones in that the periods of acclimatization were far greater, being 119 to 131 days for the "cold" fishes and 60 to 87 days for the "warm" ones. KCN was used in a 1/3,000 M solution in sea water. In 3 of these experiments, the fishes were taken directly from the temperature tanks, or the tests were made after an interval of 3 hours or less after removal. In the other 4, they were kept at a common temperature for one to two days before being subjected to cyanide. In all cases, the tests were made at "intermediate" temperatures (probably always between 18° and 20°).

Five of these 7 experiments permit of comparison between fishes of "cold" and "warm" history (7 individuals of each). The mean survival period for the former was approximately 79 minutes, that for the latter was well over 123 minutes (see foot-note 6). In two experiments in which "intermediate" and "warm" fishes were compared, the figures were about 96 and 160 minutes, respectively.

It is of interest that whereas the maximum difference in susceptibility to urethane (at a common temperature) was nearly or quite reached after a period of a day or two at the contrasted temperatures, conclusive differences in susceptibility to cyanide were not revealed until a much longer period had elapsed. Furthermore, experiments with de-oxygenated water gave results similar to those with cyanide.

In these experiments, the fishes were placed in 500-cc. flasks, filled with sea water which had been boiled or subjected to evacuation with an air-pump. Care was taken that air bubbles should be excluded and that all flasks used in any one experiment should be maintained at the same temperature.

In four experiments with *Fundulus,* the results of two in which the fishes had been acclimatized for 35 days were in conformity with those of the cyanide experiments with gobies in which long-time acclimatization had been practised. In the other two, in which the fishes had been subjected to temperature differences for 2 or 3 days, the "warm" fishes actually succumbed before either the "cold" or "intermediate" ones.

Two experiments with *Gillichthys* (6 fishes from each tank), in which the acclimatization periods were relatively brief (8 to 14 days), gave contradictory results. The mean survival times for the two in minutes were: "cold," 161; "intermediate," 178; "warm," 154.

In three experiments, on the other hand (7 "cold," 7 "warm" fishes) in which the acclimatization periods were much longer (62 to 87 days for "cold," 121 to 146 for "warm"), the mean survival times were 110 and 145 minutes, respectively. In another experiment, furthermore, in which 2 "intermediate" and 2 "warm" fishes were compared, the mean figure for the former was 137, that for the latter, 210. It may be added that in three of the foregoing experiments in which the matter was tested, all of the "warm" fishes recovered when returned to running sea water, while none of the "cold" or "intermediate" fishes did so.

Thus, as in the case of the experiments with cyanide, fishes which had been subjected to the temperature differences for considerable periods (one to several months) showed marked differences in their powers of resistance, while those subjected for much briefer periods (two weeks or less) gave results which were indecisive or mutually contradictory.

One extremely instructive experiment was performed in which heat was employed as a lethal agent. Five "cold," 5 "intermediate," and 4 "warm" gobies were used (the entire available stock at the time). The first had been in the "cold" tank for 146 days, the last in the "warm" tank for 87 days, while the "intermediate" ones had been kept for many months in natural sea water, subject only to seasonal changes of temperature (18.5° ± at the time of this experiment). The high and low temperatures were discontinued, and the fishes brought to a common temperature (18.5°) two days before the test of endurance to heat was commenced. For the latter purpose, the temperature to which all the fishes were subjected was rapidly raised, so that it reached 33° in 45 minutes. The five fishes of "cold" history succumbed almost simultaneously in 67 to 69 minutes, the temperature being 35.6° at the time. Those of "intermediate" history all succumbed (also simultaneously) in 106 minutes, the temperature having risen to 38°. The four fishes of warm history were living one and a half hours later, by which time the temperature had risen to 39.3°. This was then lowered somewhat, falling to a minimum of 36° during the night. Upon being raised to the same maximum in the morning, the fishes died within another half hour.

From the foregoing experiments the inference seemed warranted that differences in the temperature history of the fishes concerned resulted in differences in metabolic rate, which manifested themselves both in the visible rate of respiration of these fishes and in their susceptibility to certain anesthetics and lethal agents. It seemed worth while, accordingly, to test the effect of some drug which was known to accelerate oxidative metabolism in other animals. For this purpose, 2, 4-dinitrophenol was used in a concentration of 2 per cent of a saturated solution in sea water.[7]

---

[7] This substance is but slightly soluble in water. Even a saturated solution is extremely dilute.

Gobies which had been kept in running sea water (about 19° at the time) were used for these tests. Without describing these experiments in detail, it may be said that fishes kept for 16 hours in the d-n-p solution were markedly more susceptible to urethane than controls taken from pure sea water. Since, however, the d-n-p had a somewhat toxic effect, even at this low concentration, it is far from certain that the greater susceptibility of the fishes thus treated was due to heightened metabolism.

Summary and Conclusions

The experiments herein discussed were made upon two species of teleost fishes, *Fundulus parvipinnis,* and *Gillichthys mirabilis.* The anesthetics used were urethane, chloretone, and ether, the lethal agents NaCN, KCN, asphyxiation, and excessive temperature.

Fishes which had been kept at widely different temperatures, when tested at these temperatures, were found to differ markedly in the rate of their visible respiratory movements, just as they were already known to differ in their rate of oxygen consumption. Other things equal, these are higher at higher temperatures than at lower ones. Susceptibility to the single drug used in this connection (urethane) was also higher at the higher temperatures.

When fishes which had become acclimatized to high and low temperatures, respectively, were transferred to a common temperature (usually an intermediate one), those from the warmer water displayed a lower respiratory rate and a lower susceptibility to the anesthetics and lethal agents than those from the colder water. Thus the differences in physiological activity which had been originally induced by acclimatization to these differing temperatures were completely reversed when the fishes were brought to a common temperature. After this transfer, it was the former "warm" fishes which had the lower respiratory rate, etc., and the former "cold" fishes which had the higher. Fishes kept at intermediate temperatures remained intermediate.

Acclimatization to a high temperature appears to consist, in part at least, of a change whereby an initial great increase in metabolic rate is followed by a regulative process, with continued sojourn in warm water, and, conversely, that acclimatization to a low temperature involves a similar process working in the opposite direction.

While the degree of acclimatization must be, within limits, a function of time, it is surprising that after a period of one day (possibly even less) at contrasted temperatures, the maximum difference in susceptibility to urethane, when determined at a common temperature, seems to have been nearly or quite reached. When cyanides and oxygen-free water were used, on the other hand, conclusive differencs were not revealed until after a month or more of acclimatization. It may be that susceptibility to these different agents rests upon a somewhat different basis.

It is likewise noteworthy that the induced differences persisted, in one case at least, as much as 14 days after withdrawal of the environmental cause.

Rather limited experiments with dinitrophenol, a substance known to increase the metabolic rate of some animals, showed that this drug brought about a considerably increased susceptibility to urethane. It is not certain, however, that this result was due merely to an increase in metabolic rate.

LITERATURE CITED

Behre, Ellinor H., 1918. An experimental study of acclimation to temperature in Planaria dorotocephala. *Biol. Bull.,* **35**: 277.

Child, C. M., 1913. Studies on the dynamics of morphogenesis and inheritance in experimental reproduction. V. *Jour. Exper. Zool.,* **14**: 153.

Davenport, C. B., and W. E. Castle, 1895. Studies in morphogenesis, III. On the acclimatization of organisms to high temperatures. *Arch. f. Entw.,* **2**: 227.

Hathaway, E. S., 1928. Quantitative study of the changes produced by acclimatization in the tolerance of high temperatures by fishes and amphibians. *Bull. Bur. Fish.,* **43**: 169.

Loeb, J., and H. Wasteneys, 1912. On the adaptation of fish (*Fundulus*) to higher temperatures. *Jour. Exper. Zool.,* **12**: 543.

Wells, N. A., 1935. The influence of temperature upon the respiratory metabolism of the Pacific killifish, Fundulus parvipinnis. *Physiol. Zool.,* **8**: 196.

Wells, N. A., 1935a. Variations in the respiratory metabolism of the Pacific killifish, Fundulus parvipinnis, due to size, season, and continued constant temperature. *Physiol. Zool.,* **8**: 318.

Wells, N. A., 1935b. Change in rate of respiratory metabolism in a teleost fish, induced by acclimatization to high and low temperature. *Biol. Bull.* (in press).

THE MELANOPHORE–DISPERSING PRINCIPLE IN THE HYPOPHYSIS OF FUNDULUS HETEROCLITUS

L. H. KLEINHOLZ

(*From the Biological Laboratories, Harvard University, and the Marine Biological Laboratory, Woods Hole, Massachusetts*)

The numerous studies of the pigmentary changes of *Fundulus* made by Parker and his students at these laboratories, and similar studies on other fishes made by various European investigators (Pouchet, 1876; von Frisch, 1911) have, in the main, led to the conclusion that in the teleosts the ability of background adaptation depends on the nervous system.

The rôle of the pituitary gland in teleost color change has not yet been conclusively established. The responses of the melanophores of fishes to various commercial preparations of the pituitary gland leave much to be desired in the way of convincing and decisive results. Abolin (1925) reported dispersion of the melanophores of *Phoxinus* following injection of "infundin," a commercial preparation of the posterior lobe used in obstetrics. Hewer (1926) found that extracts of the posterior lobe of the cod pituitary gland produced the concentrated state of the melanophores in *Phoxinus* and that "infundin" gave inconclusive results. Giersberg (1930), on the other hand, was able to confirm Abolin with regard to the dispersing effect produced by "infundin." Przibram (1932) accounted for the differences between the results of Abolin and those of Hewer as due to differences in dosage. Spaeth (1918) and Wyman (1924) both found that commercial obstetrical "pituitrin" produced the concentrated condition of the melanophores in *Fundulus;* the former used isolated scales as test material and the latter made intraperitoneal injections.

Odiorne (1933), using several pituitary extracts prepared for clinical use, was unable to confirm Spaeth and Wyman. He placed isolated *Fundulus* scales in solutions of "pituitrin" but could not obtain the melanophore-concentration reported by Spaeth. Similarly, the injection of "pituitrin" intraperitoneally into dark- and light-adapted specimens produced no change in color, the results thereby differing from those of Wyman. By using "antuitrin," an extract of the anterior lobe of the hypophysis, Odiorne obtained the following interesting results: intraperitoneal injection of this extract brought about the concentrated state of the melanophores of dark-adapted *Fundulus* kept on a black background. The response was prompt but irregular in that it did not affect all the melanophores, and lasted not more than two hours. When "antuitrin" was added to a small amount of Ringer's solution in which isolated scales had been placed, the expanded melanophores contracted within a few minutes. The results with "antuitrin" are thus similar to those obtained by Spaeth and by Wyman, who both used "pituitrin" of the same lot. Odiorne therefore concluded that Spaeth, in presenting a new method for the standardization of pituitary extracts, was in truth doing little more than "measuring their impurity with regard to material from the anterior lobe."

There are a number of criticisms to be made of these investigations with pharmaceutical preparations. First, the use of commercial extracts of the posterior pituitary is to be discounted in the delicate qualitative tests of pigmentary reactions because such preparations probably contain the chromatophorotropic hormone only as an impurity from the intermediate lobe. It has been conclusively demonstrated from the work of Smith (1916), Allen (1916), Atwell (1919, 1934), and Swingle (1921), that the intermediate lobe of the pituitary is the localized region for the production of the melanophore-stimulating hormone in amphibia. Noble and Bradley (1933) have similarly shown that lizards, completely deprived of their intermediate lobes, are incapable of color changes. Zondek and Krohn in a series of papers in 1932 reported the isolation of "intermedin," the specific chromatophore-activating hormone, from the intermediate lobes of the pituitaries of several animals. It is probable that commercial preparations of the posterior lobe vary in their content of the chromatophorotropic hormone according to the methods of extraction and purification used.

Secondly, the solvent media and preservatives employed in these preparations may affect melanophore activity. For example, the Parke, Davis and Company obstetrical "pituitrin" is made up in 0.5 per cent chloretone. Adequate control experiments for some of the above investigations should have been made with similar injections of chloretone rather than with Ringer's solution, especially since Wyman reported that chloretone caused a dispersion of melanin granules in the pigment cells of *Fundulus*. "The reaction (of melanophores) can also be produced by various non-specific substances contained in organ-extracts, and by quinine, curarine, choline, acetylcholine, caffeine, and various preservatives" (Zondek, 1935).

Thirdly, the use of isolated scales as test objects is not a satisfactory method. The results obtained from such experiments do not necessarily imply that the mechanisms involved in the melanophore changes of the isolated scales also apply to the intact animal. Additional factors which may play a significant part in the reactions of isolated melanophores are slight salt impurities in the Ringer's solution, hypo- or hypertonicity, and oxygen deficiency of the solution in which the scale is immersed.

The treatment with pituitary extracts reviewed in the above studies is thus seen to offer no decisive conclusion as to the part played by this hormone in the color changes of *Fundulus*. The alternate approach to this problem is that involving ablation of the hypophysis. Matthews (1933) successfully hypophysectomized *Fundulus* and noted that this operation had no effect on the pigmentary responses, since such operated fishes underwent normal color changes. However, the pigment granules in the melanophores became *concentrated* when he immersed an isolated scale in an extract of one *Fundulus* pituitary ground up in 0.05 cc. of N/10 NaCl. From this Matthews concluded that the hypophysis of *Fundulus* does contain the melanophore-activating principle.

In view of the criticisms made of the methods involving the use of isolated scales, it was thought advisable to test the chromatophorotropic activity of *Fundulus* pituitaries on a number of animals which are known to have definite melanophore responses to this hormone. This problem was undertaken at the suggestion of Professor Alden B. Dawson to whom I am indebted for many helpful criticisms and kind suggestions. Dr. A. A. Abramowitz also very kindly gave of his time and advice.

MATERIALS AND METHODS

The animals used as test objects in this investigation were *Fundulus heteroclitus* (the common killifish), *Ameiurus nebulosus* (catfish), *Rana pipiens*, and *Anolis carolinensis* (the American "chameleon"). Extracts of the *Fundulus* pituitaries were made by grinding the glands with a glass rod in a Syracuse dish the bottom of which had been roughened with powdered emery. Solutions of different concentrations were made of these glands in cold-blooded Ringer's and were injected intraperitoneally.

The normal color reactions of the test animals are considered under each sub-title.

Anolis carolinensis

This lizard is the so-called "chameleon" found in our southern states. Specimens obtained from a dealer in Georgia were caged and kept in a warm room. Feeding with blow-flies and daily sprinkling of the cages with water maintained the animals in excellent condition.

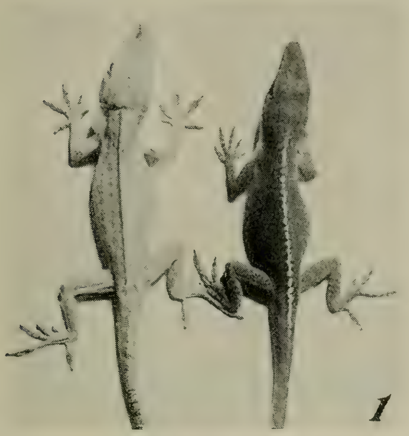
These lizards are brown at room temperature (18°–28° C.) in the light, because the melanophores are in the dispersed condition. In darkness the animals are green because the melanin granules have retracted from immediately beneath the epidermis into the primary and larger branches of the melanophores, thus exposing the yellow oil-droplet layer; light, which is reflected from the leucophore layer as blue, mixes with the yellow rays from the oil-droplet layer and so appears green at the surface (von Geldern, 1921). If dark animals are placed upon a brightly-illuminated white background they gradually turn green. The time for this change requires from 10 to 30 minutes, but varies with different specimens. Conversely, a light animal placed on an illuminated black background darkens, but the reaction is more rapid, and takes place in from 5 to 15 minutes. Ablation of the anterior lobe of the pituitary does not interfere with the normal color changes, but those specimens from which the intermediate lobes have also been removed lose the ability to darken on backgrounds which normally evoke the dark condition, and remain permanently in the green state. Fig. 1 shows the striking difference in color between a normal and an hypophysectomized specimen.

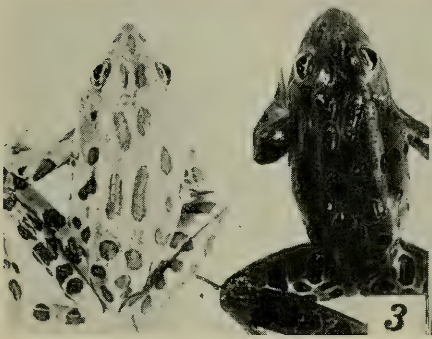
Three normal lizards were placed in the dark-room for an hour. At the end of this time they were green in color. Two were each injected with 0.25 cc. of an extract of 4 *Fundulus* pituitaries in 0.5 cc. of Ringer's; the third animal was injected with 0.25 cc. of brain tissue suspension. The following table shows the results:

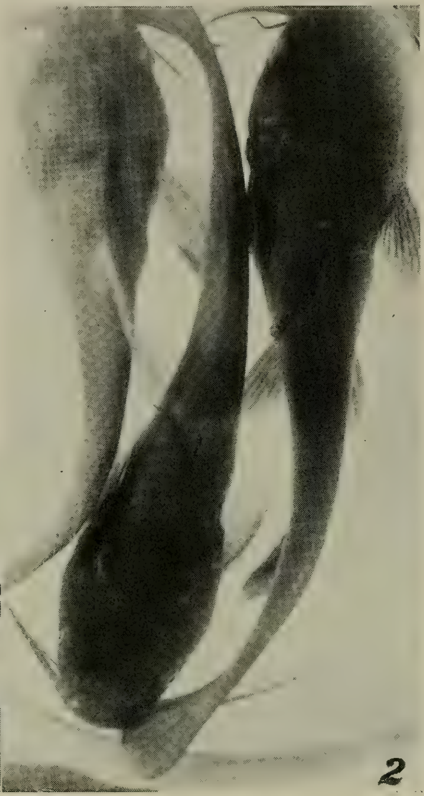
	Pituitary	Pituitary	Brain
At injection	Green	Green	Green
20 minutes after	Brown	Brownish-green	Green
45 minutes after	Brownish-green	Brownish-green	Green
60 minutes after	Brownish-green	Green	Green
80 minutes after	Green	Green	Green

The two animals injected with the pituitary extract darkened, whereas the control remained unaffected. The rapid fading of the induced dark condition was due to the small amount of pituitary tissue injected. When 8 *Fundulus* pituitaries in 0.4 cc. of Ringer's were injected into an animal that had been adapted to darkness for an hour and was consequently in the green condition, the brown color response was obtained in 12 minutes and persisted, even though the animal remained in the dark-room, for over 6 hours.

One hypophysectomized lizard which had remained green for more than 10 weeks was injected with 0.25 cc. of 4 *Fundulus* pituitaries in 0.30 cc. of Ringer's. The animal turned brown after 3 minutes and remained dark for more than 12 hours, having been kept all the time on a brightly illuminated white background.


1


3


2

EXPLANATION OF PLATE I

FIG. 1. On the right is a normal *Anolis* in the dark condition. The animal on the left has been hypophysectomized and is consequently in the green or light state.

FIG. 2. On the right is a normal catfish that has been darkened by adaptation to a black background. The fish in the center had been light-adapted to a white background and then been injected with 12 *Fundulus* pituitaries; one hour after the injection it was photographed in this dark condition. The animal on the left is a control light-adapted fish that had received 0.5 cc. of Ringer's solution intraperitoneally.

FIG. 3. The frog on the right had been light-adapted and then injected with 8 *Fundulus* pituitaries, photographed 75 minutes after the injection. The animal on the left was the control that received an injection of Ringer's solution.

Two specimens, adapted to darkness, were injected each with 0.20 cc. of Ringer's solution. They showed no color change.

Rana pipiens

Four frogs were placed on an illuminated white background for 24 to 48 hours. At the end of this time they were maximally pale, the web melanophores being in the punctate condition. Three of these frogs were injected with extracts of *Fundulus* pituitaries, so that each received the equivalent of 8 fish glands; the fourth animal was injected with Ringer's solution. The animals which received the pituitary began to darken within 15 minutes and after an hour were extremely dark, the web melanophores being reticulate (Fig. 3). The dark condition persisted for 3 to 4 hours although the animals remained on the illuminated white background. The control specimen showed no change in color.

Ameiurus nebulosus

These fishes become light greenish on a white background and very dark brown or black on black backgrounds. Of three animals adapted to a white background, one was injected with 8, and a second with 12 macerated *Fundulus* pituitaries, while the third received 0.5 cc. of Ringer's solution. The fishes receiving the pituitary injections began to darken slightly after 30 minutes and at the end of one hour were perceptibly much darker than the control (Fig. 2). In both animals

---

EXPLANATION OF PLATE II

The photographs on this plate were very kindly taken for me by Mr. E. P. Little, on the staff of the Marine Biological Laboratory.

FIG. 4. The band in the caudal fin of a *Fundulus;* this region has faded, 9 days after the denervating cut, as a result of keeping the fish in an illuminated white dish. The original band is shown outlined in ink.

FIG. 5. Detail of a region in the band of Fig. 4, showing the melanophores in the punctate condition.

FIG. 6. Darkening of a faded denervated band induced by the injection of *R. pipiens* pituitary tissue.

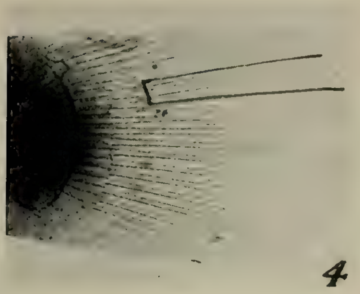
FIG. 7. Detail of the band in Fig. 6, showing the melanophores in varying degrees of dispersion. At the top of the photograph is shown an innervated region of the tail with the melanophores punctate.

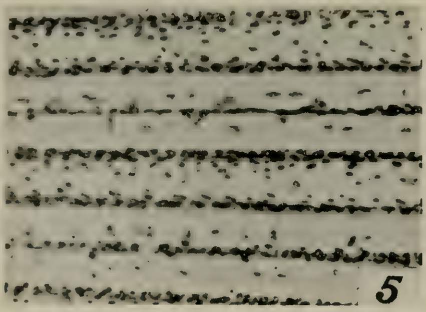
FIG. 8. Slight darkening of the faded band resulting from the injection of *Fundulus* pituitaries.

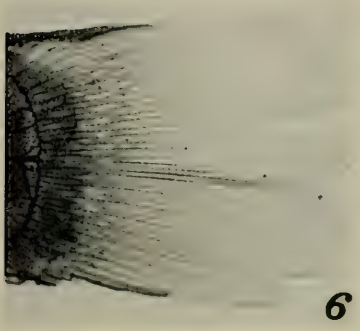
FIG. 9. Detail of a portion of the band in Fig. 8. Some of the melanophores show stellation.

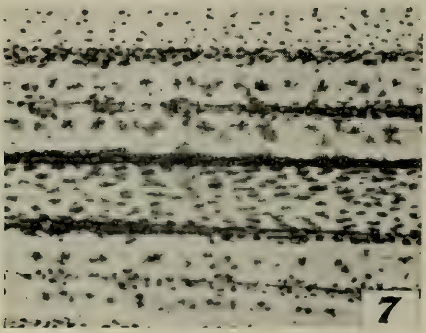
FIG. 10. A darkening of the band provoked by the injection of Parke, Davis and Company obstetrical "pituitrin."

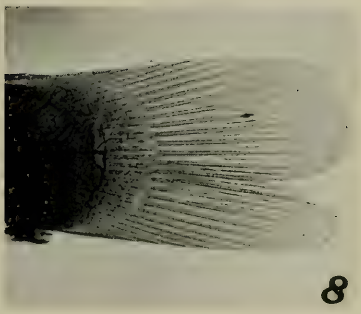
FIG. 11. Low power microscopic view of a portion of the band in Fig. 10, showing the extreme dispersion of the melanophores. At the bottom of the print is a portion of an innervated region showing the melanophores in the punctate condition.

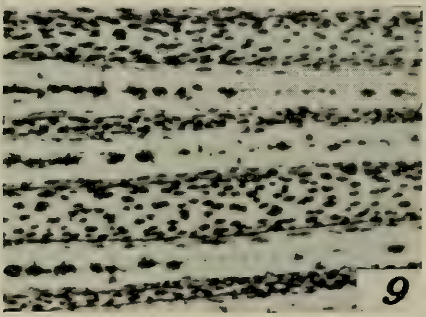

4

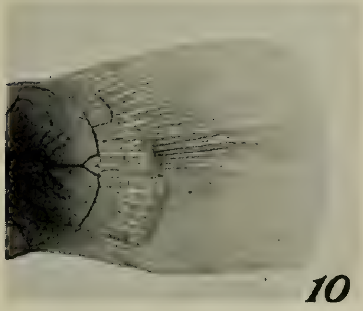

5


6


7


8


9


10

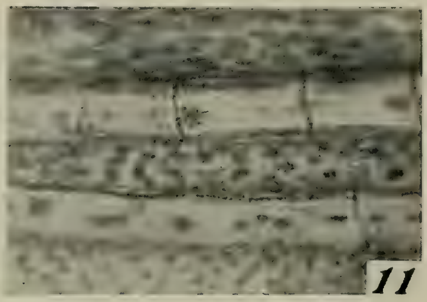

11

PLATE II

thus treated, however, the dark condition attained was not as pronounced as that obtained normally by adaptation to a black background. The control catfish remained in the light condition.

Fundulus heteroclitus

Specimens were placed in white or black dishes and after 24 hours were fully adapted to their backgrounds, the animals in the white dish being light, and those in the black dish, dark. In the first test, one dark and one light fish each received 0.25 cc. of extract of 2 *Fundulus* pituitaries in 0.5 cc. of Ringer's. The fishes showed no color changes for two hours.

In a second test, a suspension of 5 *Fundulus* pituitaries was injected into a light killifish and a similar solution into a dark fish. There was no color change in either during three hours of observation.

In the third test, the equivalent of 10 hypophyses was introduced into a light and into a dark *Fundulus*. For the following 90 minutes the melanophores remained unaffected by this concentrated extract.

The work of Matthews shows that ablation of the hypophysis does not affect the normal color changes of *Fundulus*. He believed that the pituitary of this fish did contain the melanophore-activating principle since it caused a *concentration* of the melanin in the pigment cells of an isolated scale that was immersed in an extract of the gland. The fact that the melanophores of those vertebrates, which respond to the pituitary pigmentary hormone, do so by a *dispersion* of the melanin granules, would imply either that the melanophore reactions of *Fundulus* differ from those of other vertebrates or that the isolated scale technique is at fault.

Although hypophyseal extracts from *Fundulus heteroclitus* are capable of dispersing the pigment within the melanophores of a fish, an amphibian, and a reptile, the melanophores of *Fundulus* itself remain unaffected by such extracts. Both Hewer and Matthews have offered the suggestion that such lack of response might be due to the secretion of sub-minimal amounts of the pigmentary hormone in the normal animal. I have found relatively concentrated extracts of 5 or 10 glands to have no observable effect on the melanophores.

The possibility appeared that the failure of the melanophores of *Fundulus* to respond to its own demonstrably-active pituitary gland might be due to some masking action of the nervous system. To test this, denervated bands were made in the caudal fins of *Fundulus* according to the method used by Wyman (1924). This consists of making a transverse incision across the fin rays from one surface of the tail to the other surface. As a result, the radiating nerve fibers of that region are cut and a denervated band of melanophores is formed extending from the cut distally to the end of the caudal fin. The pigment in the area distal to the cut becomes maximally dispersed within several minutes. Parker (1934*a*) attributes the darkening of this band to the mechanical stimulation of the dispersing nerve fibers at the time of making the incision. Degeneration of the nerves proceeds posteriorly from the incision and regeneration from the nerves anterior to the cut does not begin until about 18–20 days after the initial operation (Parker and Porter, 1933). There is thus a period of several days when the melanophores in such bands are without physiological nerve supply.

Three specimens of *Fundulus* with prepared denervated bands were placed in white dishes. When examined on the ninth day after the operation the bands were completely faded, the melanophores in these areas being punctate, as were also the melanophores in the unoperated regions of the fishes (Figs. 4 and 5). Into each of these animals was injected a suspension of 5 neuro-intermediate lobes of frog hypophyses in Ringer's solution. The bands darkened in from 10 to 20 minutes; microscopic examination showed that the melanophores in these areas had their pigment dispersed, some to the maximum amount and others to a stellate condition (Figs. 6 and 7). The melanophores in the innervated regions of the caudal fins remained punctate.

This experiment was repeated with extracts prepared from the pituitary glands of *Fundulus*. The procedure was to note the condition of the melanophores in a definite region of the band, make the injection, immediately afterwards return the animal to its illuminated white dish, and at varying intervals to examine the same region microscopically. The protocols for 5 experimental fishes best describe the results:

Animal No. 2.
June 22. Caudal band denervated. Placed in white dish.
July 2. Band has faded.
10:40 A.M.—most of the melanophores in the band are punctate, some are very slightly stellate.
Injected 0.20 cc. of 5 *Fundulus* pituitaries in 0.25 cc. of Ringer's.
11:00 A.M.—band has darkened; the melanophores in the denervated area are pronouncedly stellate; those in the innervated areas are punctate.

Animal No. 6.
June 24. Female, band prepared. Placed in white dish.
July 4. 11:30 A.M.—band completely faded, melanophores punctate.
11:34 A.M.—injected 0.20 cc. of 8 *Fundulus* pituitaries in 0.25 cc. of Ringer's.
11:45 A.M.—band darkening, many melanophores show beginning stellation.
11:55 A.M.—most of the melanophores are slightly stellate; a few are completely dispersed.

Animal No. 7.
June 24. Male, operated and placed in white dish.
July 5. 3:44 P.M.—band faded, melanophores punctate. Injected 0.20 cc. of 8 *Fundulus* pituitaries in 0.25 cc. of Ringer's.
3:55 P.M.—no change.
4:05 P.M.—no change.
4:15 P.M.—no change.

Animal No. 8.
June 27. Female, operated and placed in white dish.
July 5. 10:05 A.M.—band faded, melanophores punctate.
10:07 A.M.—injected 0.15 cc. of 4 *Fundulus* glands in 0.20 cc. of Ringer's.
10:20 A.M.—slight initial stellation in the band.
10:30 A.M.—some of the melanophores are slightly stellate.

Animal No. 10.
June 27. Female, band denervated, placed in white dish.
July 10. 11:33 A.M.—band faded, melanophores punctate.
11:35 A.M.—injected 0.13 cc. of 8 *Fundulus* pituitaries in 0.15 cc. of Ringer's.
11:42 A.M.—band darkening, melanophores of the band are slightly stellate.
11:55 A.M.—band dark; a few of the melanophores are completely dispersed, many show slight stellation, and some remained punctate.

In these tests, denervated melanophores responded to pituitary injections by a dispersion of their pigment granules (Figs. 8 and 9). Not all of the melanophores in the band reacted to this substance, and not all were affected to the same extent.

In a third series of tests, five *Fundulus heteroclitus* females with faded denervated bands (9 and 10 days after the denervating cuts) were each injected with 0.20 cc. of Parke, Davis and Company obstetrical "pituitrin" (ampoules no. 3018380 and no. 3064766). The bands darkened after 10 minutes and microscopic examination showed the melanophores of those regions to be in a condition of extreme dispersion (Figs. 10 and 11). As Odiorne has previously reported (1933), the "pituitrin" had no effect on the remaining innervated melanophores of the tail and body. Three of these injected animals were kept under observation for two hours. At the end of this time the band of one fish had faded only very slightly while those of the remaining two specimens persisted unfaded. Because this pituitary solution contains 0.5 per cent chloretone, added as a preservative, controls were run on three fishes possessing faded caudal bands. Intraperitoneal injections of 0.20 cc. of 0.5 per cent chloretone dissolved in amphibian Ringer's solution were made. Neither the denervated bands nor the innervated melanophores showed any change, all remaining in the punctate condition. Wyman (1924) found that when a 1 per cent chloretone solution was siphoned over the gills of *Fundulus*, the innervated melanophores very soon became dispersed, while the melanophores in the faded caudal bands remained punctate or slightly stellate. The discrepancy between Wyman's and my results may be due to a rapid elimination or destruction of the drug by the body fluids.

The response of denervated melanophores to hypophyseal extracts suggests that the pituitary secretion of *Fundulus* may be a humoral factor to be considered in those studies of color changes where the method of denervated regions is used. In this connection, the study made by Parker (1934*b*) on the catfish, *Ameiurus*, is of interest. He found that the pituitary gland of this fish contains the melanophore-dispersing principle. Hypophysectomized catfishes, however, undergo the same color changes on black or white backgrounds that normal unoperated specimens do. When a catfish in the light state with a faded denervated band is put into a black dish, the light band becomes dark approximately at the same time as the whole fish does. When light fishes with faded bands are hypophysectomized and then placed in black dishes, the bands remain light for several hours, after which they darken. This difference in the darkening of the denervated bands Parker attributes to the pituitary hormone in the blood of the normal catfish and to its absence from the hypophysectomized animal. The final darkening of the band in the operated fishes he believes due to the diffusion into the band of a dispersing neurohumor from the adjacent nerve terminals. Whether *Fundulus heteroclitus* will react in similar fashion to hypophysectomy remains to be investigated.

SUMMARY

1. From these experiments it appears that the hypophysis of *Fundulus heteroclitus* contains a hormone which is capable of dispersing the melanin granules in the pigment cells of the catfish, the frog, and the lizard.

2. The innervated melanophores of *Fundulus* are unresponsive to the pigmentary hormone of their own pituitary glands, even though concentrated extracts were injected, and are also unresponsive to extracts from amphibian and mammalian pituitaries.

3. This lack of response of the normal innervated melanophores appears to be due to some nervous control, because denervated melanophores respond to pituitary injections by a typical *dispersion* of their pigment. This result is in contrast to that reported by Matthews, who used isolated scales.

LITERATURE CITED

ABOLIN, L., 1925. Beeinflussung des Fischfarbwechsels durch Chemikalien. I. Infundin- und Adrenalinwirkung auf die Melano- und Xanthophoren der Elritze (Phoxinus lævis Ag.). *Arch. mikr. Anat. Entw.-mech.*, **104**: 667.

ALLEN, B. M., 1916. The results of extirpation of the anterior lobe of the hypophysis and of the thyroid of Rana pipiens larvæ. *Science*, **44**: 755.

ATWELL, W. J., 1919. On the nature of the pigmentation changes following hypophysectomy in the frog larva. *Science*, **49**: 48.

ATWELL, W. J., 1934. Intentional production of silvery tadpoles which metamorphose. *Anat. Rec.*, **58**: 48 (abstract).

VON FRISCH, K., 1911. Beiträge zur Physiologie der Pigmentzellen in der Fischhaut. *Arch. ges. Physiol.*, **138**: 319.

VON GELDERN, C. E., 1921. Color changes and structure of the skin of Anolis carolinensis. *Proc. Calif. Acad. Sci.*, **10**: 77.

GIERSBERG, H., 1930. Der Farbwechsel der Fische. *Zeitschr. vergl. Physiol.*, **13**: 258.

HEWER, H. R., 1926. Studies in colour changes of fish. I. The action of certain endocrine secretions in the minnow. *Brit. Jour. Exper. Biol.*, **3**: 123.

MATTHEWS, S. A., 1933. Color changes in Fundulus after hypophysectomy. *Biol. Bull.*, **64**: 315.

NOBLE, G. K., AND H. T. BRADLEY, 1933. The relation of the thyroid and the hypophysis to the molting process in the lizard, Hemidactylus brookii. *Biol. Bull.*, **64**: 289.

ODIORNE, J. M., 1933. The effects of the pituitary hormones on the melanophores of fishes. *Proc. Nat. Acad. Sci.*, **19**: 745.

PARKER, G. H., 1934*a*. Prolonged activity of momentarily stimulated nerves. *Proc. Nat. Acad. Sci.*, **20**: 306.

PARKER, G. H., 1934*b*. Color changes in the catfish Ameiurus in relation to neurohumors. *Jour. Exper. Zoöl.*, **69**: 199.

PARKER, G. H., AND H. PORTER, 1933. Regeneration of chromatophore nerves. *Jour. Exper. Zoöl.*, **66**: 303.

POUCHET, G., 1876. Des changements de coloration sous l'influence des nerfs. *Jour. de l'Anat. et de Physiol.*, **12**: 1.

PRZIBRAM, H., 1932. Einfluss von Infundin- und Adrenalindosierung auf die Färbung unserer Frösche, Rana esculenta L., R. fusca Rösel, Hyla arborea L. *Zeitschr. vergl. Physiol.*, **17**: 565.

SMITH, P. E., 1916. The effect of hypophysectomy in the early embryo upon the growth and development of the frog. *Anat. Rec.*, **11**: 57.

SPAETH, R. A., 1918. Concerning a new method for the biological standardization of pituitary extract and other drugs. *Jour. Pharm. Exper. Therap.*, **11**: 209.

SWINGLE, W. W., 1921. The relation of the pars intermedia of the hypophysis to pigmentation changes in anuran larvæ. *Jour. Exper. Zoöl.*, **34**: 119.

WYMAN, L. C., 1924. Blood and nerve as controlling agents in the movements of melanophores. *Jour. Exper. Zoöl.*, **39**: 73.

ZONDEK, B., 1935. Chromatophorotropic principle of the pars intermedia of the pituitary. *Jour. Am. Med. Assoc.*, **104**: 637.

ZONDEK, B., AND H. KROHN, 1932*a*. Hormon der Zwischenlappens der Hypophyse. I. Die Rotfärbung der Elritze als Testobjekt. *Klin. Wschr.*, **11**: 405.

ZONDEK, B., AND H. KROHN, 1932*b*. Hormon der Zwischenlappens der Hypophyse. II. Intermedin im Organismus (Hypophyse, Gehirn). *Klin. Wschr.*, **11**: 849.

ZONDEK, B., AND H. KROHN, 1932*c*. Hormon der Zwischenlappens der Hypophyse. III. Zur Chemie, Darstellung und Biologie des Intermedins. *Klin. Wschr.*, **11**: 1293.

RENAL FUNCTION IN MARINE TELEOSTS

I. URINE FLOW AND URINARY CHLORIDE

ALLAN L. GRAFFLIN[1]

DEPARTMENT OF ANATOMY, HARVARD MEDICAL SCHOOL

Studies on renal function in the fishes have brought out some exceedingly interesting facts which have been of considerable value in the general problem of kidney function (for review of the literature see Marshall, 1934) and justify a more extended study along these lines. As a necessary basis for further physiological work, it was essential to obtain information concerning the normal rate of urine flow in the marine teleosts. The reports available in the literature were highly variable (Burian, 1909; Edwards and Condorelli, 1928; Marshall, 1930; Smith, 1930); and the theoretical considerations of Smith (1930) demanded a very low urine flow in marine teleosts as compared to fresh-water species.

In a previous investigation (Grafflin, 1931) we were led to the conclusion that the normal urine flow of the glomerular sculpin (*Myoxocephalus octodecimspinosus*) is probably below 4.0 cc., and of the aglomerular toadfish (*Opsanus tau*) is probably below 2.5 cc. per kilogram per 24 hours. It was further concluded that the normal urine of both species is almost invariably free of chloride and relatively high in total nitrogen. When these animals were followed under the usual experimental conditions, they were found to develop a marked diuresis, with the appearance of large quantities of chloride in the urine, a rapid diminution in the urinary total nitrogen, and an elevation of the plasma chloride. With the use of extreme precautions to avoid skin injury, it was possible to obtain low urine flows and zero (or trace) urinary chlorides in the majority of animals. Some animals with low flows, however, showed a rather high concentration of urinary chloride; while a number of animals showed a relatively high rate of flow, which was sometimes associated with a fairly high urinary chloride, but at other times with a low, or even zero, chloride.

---

[1] Some of the data reported in this paper were obtained while I was a Fellow of the John Simon Guggenheim Memorial Foundation (1934). I wish to express my gratitude to Prof. R. Dohrn, Director of the Stazione Zoologica, Naples, Italy, and to Dr. E. J. Allen, Director of the Laboratory of the Marine Biological Association, Plymouth, England, and their staffs for their generous hospitality and coöperation while I was a guest at their laboratories.

These latter findings led us to seek additional data upon the normal rate of urine flow, and to attempt to settle (1) whether there is any definite correlation between the rate of urine flow and the urinary concentration of chloride; and (2) whether the urinary excretion of chloride in the marine teleosts may be considered a normal physiological process, or whether it is invariably to be interpreted as a criterion of abnormality. In the experiments upon urine flow, the length of the urine collection period was varied in an attempt to determine whether some factor such as an initial diuresis or anuria following catching and tying of the urinary papilla was confusing the results.

Material and Methods

The fishes used in this study were the sculpin (*Myoxocephalus octodecimspinosus*), flounder (*Pseudopleuronectes americanus*), cod (*Gadus morrhua*), goosefish (*Lophius piscatorius*), plaice (*Pleuronectes platessa*), *Orthagoriscus mola*, *Scorpæna scrofa*, *Trigla corax*, and *Zeus faber*. The sculpins and flounders [2] recorded in Figs. 1 and 2 were handled in the following manner. Immediately after being hooked each animal was brought to the surface and dipped up in a bucket. The hook was removed under water, the fingers touching only the inside of the mouth, and any fish bleeding and (or) torn at the point of hooking was discarded. The fish was then transferred without touching to a shallow porcelain bowl, where it was held under water with bare hands, and with great care to avoid skin injury and interference with respiration. The bladder was emptied by massage of the overlying abdominal wall and the urinary papilla was tied off with fine silk thread. Catheterization was not used in the preliminary emptying of the bladder for the following reasons. The urinary papilla is extremely sensitive, and insertion of the catheter frequently causes the animal to struggle vigorously, with increased skin injury as the inevitable result. Furthermore, it was desirable to eliminate any possible reflex effects which the stimulus of catheterization might have upon the kidney. At the end of the tying period the animal was killed by a blow on the head and the accumulated urine was removed from the exposed bladder by syringe. The animals were then weighed, and urine flows are expressed in terms of cc. per kilogram of fish per 24 hours. Chloride (expressed as millimols per liter) was determined by the method of Van Slyke (1923) with the modifications reported by Smith (1930). The experiments upon sculpin and flounder were carried out at the Mount Desert Island Biological Laboratory, Salsbury Cove, Maine.

---

[2] The kidneys of both species are glomerular (Nash, 1931; Marshall and Grafflin, 1932; Marshall, 1934; Clarke, 1934).

RESULTS

Sculpin [3]

The data on 83 sculpins are summarized in Fig. 1, and show a considerable variation in urine flow. Since failure to empty the bladder completely at the beginning could lead to serious error, particularly in short collection periods, we tested our ability in this regard on 22 freshly caught sculpins handled with the same precautions as the experimental animals. The residues were as follows: 8—completely empty; 11—0.02 to 0.03 cc.; 1—0.05 cc.; 1—0.32 cc.; 1—0.45 cc. In the last two animals the recorded urine flows would have been too high by about 9 cc. in 6 hours, 2.3 cc. in 24 hours. With a residue of 0.03 cc. the error would be, for a fish of 175 grams (average of series), 0.7 cc. for 6 hours, 0.17 cc. for 24 hours. The danger of incomplete emptying is further emphasized by one group of four sculpins which were emptied in the same manner by another person, and in which the residual urine on immediate sacrifice would have introduced errors of 2.8, 4.0, 4.4 and 12.0 cc. in a 6-hour period, 0.7 1.0, 1.1 and 3.0 cc. in a 24-hour period.

It is clear that incomplete emptying of the bladder at the beginning of the collection period must be regarded as a serious source of error in the sculpin. The aberrant high flows in Fig. 1 could all be easily explained on this basis, and it is certain that many of the other flows are too high, some of them by a considerable margin. Nevertheless, in view of the results obtained on the control animals, when this source of error is fully taken into account it must still be concluded that there is a marked variation in the *actual* urine flow under our experimental conditions. The relatively narrow range of variation in the longer collection periods (over 30 hours) is deceptive, since some animals tied up for such a long period showed over-distention of the bladder, and had to be discarded. The data on relatively short collection periods (6.5–8.5 hours) are particularly interesting in view of the following statement by Clarke and Smith (1932): "There is evidence in our experiments that (reflex?) anuria may occur in fish and that it may persist for some hours, particularly after the fish is first catheterized. This fact is of great importance in the measurement of 'normal' urine flows and has been overlooked by investigators in the past (compare Smith, 1930; Grafflin, 1931)" (p. 142). An examination of Fig. 1 makes it quite clear that such a period of anuria did not occur in our experimental animals. Actually, the data strongly suggest a significantly higher average rate of urine flow in the hours immediately following emptying and tying. However, we feel that without additional data the indicated "initial diuresis" cannot for the present be accepted as proved.

---

[3] I wish to express my indebtedness to Mr. David Ennis for his assistance in the sculpin and flounder experiments recorded in Figs. 1 and 2.

The urinary chloride concentrations in the same series of 83 sculpins are summarized in Fig. 2. The chloride is from 0 to 10 millimols per liter in over half of the animals (46) and is below 50 millimols per liter in over two-thirds (58). The significant fact is that the 37 sculpins showing more than a trace of chloride (over 10 millimols per liter) are scattered indiscriminately over the entire range of urine flow.

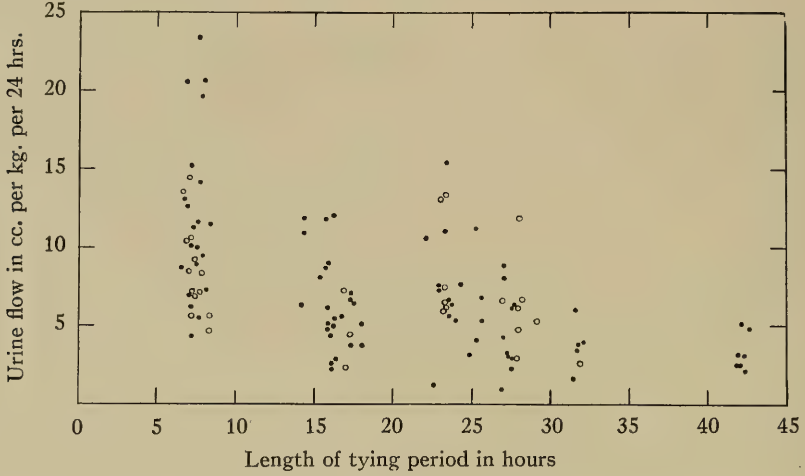


FIG. 1. Observations upon rate of urine flow in freshly-caught sculpins and flounders, with urine collection periods of variable length. Solid circles, Sculpin. Open circles, flounder.

Discussion of Sculpin Data and Conclusions

The two most important facts brought out by the present data are: (1) the wide variation in the urine flow and urinary chloride concentration in freshly caught sculpins, under experimental conditions which were as ideal and as nearly constant as could be obtained; (2) the lack of any direct relationship between the rate of urine flow and the urinary chloride concentration, which is obvious from even a casual examination of Fig. 2. Grafflin (1931), from more meager data and at a time when many of the problems involved in fish experimentation were not fully appreciated, was led to the conclusion that the normal urine flow of the sculpin is probably below 4.0 cc. per kilogram per day. The present experiments do not bear out this earlier conclusion, but indicate rather a considerable range of urine flow as a normal base-line for physiological work. The chloride data likewise question the interpretation of the presence of chloride in the urine as a criterion of abnormality, and suggest instead that urinary chloride excretion may be a normal physiological process in the sculpin. In this connection the following observations are of interest. In an earlier study (1931) we reported the chloride concentration of original urine, removed from the bladder immediately after catching with hook and line, to be zero or trace in a short series of sculpins. Pitts (1934) has more recently reported similar data upon eleven sculpins, eight of which showed zero urinary chloride, while the other three showed 53, 74 and 147 millimols per liter respectively. In four specimens of the closely related daddy sculpin (*Myoxocephalus scorpius*) Pitts obtained values of 0, 34, 48, and 128 millimols per liter. When Pitts' positive demonstration of chloride in considerable amounts in the urine of six out of fifteen freshly caught sculpins (two species) is considered in conjunction with the present data, we feel that the conclusion is probably justified that the sculpin may excrete variable, and sometimes considerable amounts of chloride in the urine under normal physiological conditions. On this basis, concurring with the conception that the majority of sculpins normally excrete an essentially chloride-free urine, the presence of chloride in the urine cannot be accepted as a criterion of abnormality without further proof, particularly since it is often found in large amounts with a very low rate of urine flow.

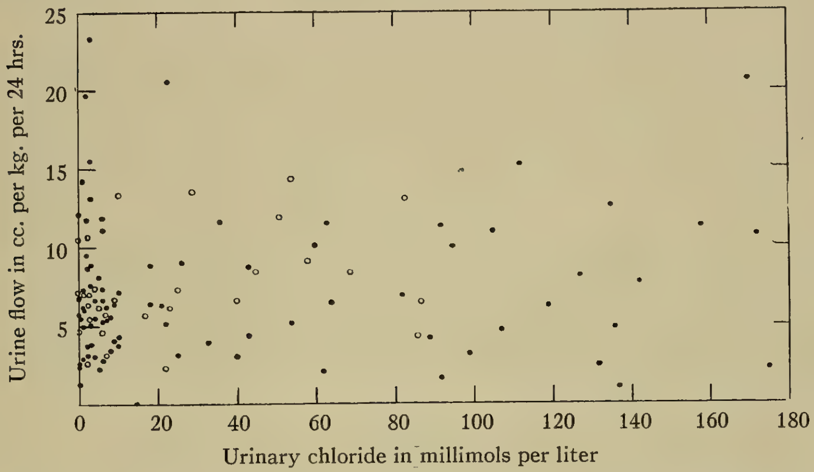


FIG. 2. Graph showing urinary chloride concentration, plotted against rate of urine flow, in the sculpins and flounders recorded in Fig. 1. Solid circles, sculpin. Open circles, flounder.

Sculpins with Pyloric Tie

Smith (1930, 1932) has demonstrated quite conclusively that marine teleosts normally ingest relatively large quantities of sea water, which undergoes absorption in the gastro-intestinal tract. Grafflin and Ennis (1934) have recently reported experiments designed for the study of urine formation when this intestinal absorption of sea water was prevented. Sculpins, freshly caught and handled with care, were anesthetized with urethane and the upper intestine was tied off at its junction with the stomach. At the end of the operation, with the animals completely relaxed by the anesthetic, the bladder was emptied by massage of the overlying abdominal wall. The urine flow and urinary chloride data obtained upon these operated animals in the ensuing short collection period (5.5–9.9 hours) are particularly interesting in connection with the data reported above. In brief, the results on 24 such sculpins were as follows: in 16 the chloride varied from 0 to 9 millimols per liter, urine flow 5.4 to 19.3 cc. per kilogram per 24 hours; in the other 8, chloride from 16 to 120 millimols per liter, flow from 6.8 to 16.0 cc. Even when incomplete preliminary emptying of the bladder as a source of error is fully taken into account, these data furnish additional evidence that in the sculpin there is no direct relationship between the rate of urine flow and the urinary chloride concentration. The wide variation in the rate of urine flow in these animals, under experimental conditions which were essentially identical, is likewise noteworthy.

Flounder

The data upon urine flow and urinary chloride in thirty flounders are included in Figs. 1 and 2. As in the sculpin, incomplete emptying of the bladder at the start of the collection period is a potential source of considerable error. However, in only one of the control animals was there any measurable residue, and in that case it was 0.1 cc. When this source of error is taken fully into account, the data demonstrate a considerable variation in the actual rate of urine flow under our experimental conditions. Furthermore, the results obtained in short collection periods (6.5–8.5 hours) make it evident that in the flounder, as in the sculpin, the manipulations involved in catching, and in tying of the urinary papilla, were not followed by an initial period of anuria. The urinary chloride concentration varies from 0 to 87 millimols per liter, with values from 0 to 10 millimols in 16 animals. From a consideration of Fig. 2, it must be concluded that in the flounder, as in the sculpin, there is no direct relationship between the rate of urine flow and the urinary chloride concentration.

Orthagoriscus mola

Advantage was taken of an opportunity to obtain specimens of the relatively rare *Orthagoriscus mola* from a large tunny-net in the Bay of Naples. The fish were removed from the net as soon as it was sufficiently close-hauled, and urine was obtained at once by syringe from the exposed bladder. In 33 specimens the urinary chloride concentration varied from 20 to 137 millimols per liter, with an even distribution over the entire range. Although, despite many attempts, we were unable to obtain a single specimen showing a chloride-free urine, the relatively low values obtained in some cases indicate the ability of this species to excrete such a urine. The question at issue is whether the presence of chloride in the urine of these fish may be attributed entirely to skin injury or other factors associated with their presence in the net. It is our belief that this is not the case, and that the urinary excretion of chloride in variable amounts is probably a normal, physiological process in *Orthagoriscus*, for the reasons given in the footnote.[4]

Scorpœna scrofa

Urine was obtained from nine specimens of *Scorpœna scrofa* which were thoroughly acclimatized to the life in the aquarium at the Stazione Zoologica in Naples. The chloride concentrations were 0, 6, 118, 143, 145, 148, 149, 155, and 163 millimols per liter. Urine was likewise removed from five specimens which had become entangled in a net (rete di posta, Stellnetz) which had been in the water eight hours before hauling. Here the chloride concentrations were 5, 36, 107, 107, and 144 millimols per liter. These data demonstrate at once the ability of the kidney of this species to elaborate a chloride-free urine. When examined in conjunction with the plasma chloride values obtained upon such specimens, the aquarium data furthermore indicate that urinary chloride excretion may well be a physiological process in

---

[4] The net was of tremendous size, and had been in the water for months. *Orthagoriscus* is a very sluggish swimmer, swam lazily about in the net with no evidence of excitement, and on no day when specimens were obtained was any other species present in the net to disturb them. The net was hauled quite rapidly, and only in the last short period of close-hauling were the fishes crowded and in contact. The bladder capacity is very large, and in the specimens taken for study the urine volume was likewise very large. Under the circumstances, it seems not unreasonable to assume that the fish were under normal conditions up to the time of hauling the net. Granting this assumption, and taking into consideration our knowledge of the variations in urine flow and chloride excretion under experimental conditions in other marine teleosts, it is inconceivable that the urine of the majority of these fish could have been chloride-free up to the last short period of hauling. Those showing low urinary chloride concentration, on the other hand, might conceivably have been chloride-free.

Scorpæna. Thus, 6 aquarium specimens showed a plasma chloride concentration varying from 147.6 to 157.9 millimols per liter, with an average of 151.3 millimols. This mean value is close to the lowest average (150.6 millimols per liter—*Gadus pollachius*) obtained in a rather extensive study of various marine teleosts bled immediately after being caught with hook and line (Grafflin, 1935). On the basis of our present knowledge, this observation apparently justifies the conclusion that the aquarium specimens of *Scorpæna* are to be considered as essentially normal. Three of these specimens, with plasma chloride concentrations of 149.6, 150.8, and 151.2 millimols per liter, gave simultaneous urinary chloride concentrations of 148, 143, and 149 millimols per liter respectively.

Miscellaneous Fishes

Single specimens of *Trigla corax* (Naples aquarium) and *Gadus morrhua* (long line—Plymouth) gave urinary chloride concentrations of 8 and 5 millimols per liter respectively, and demonstrate the ability of these two species to excrete an essentially chloride-free urine. Four specimens taken at Plymouth in a steam trawl which had been dragged for about an hour yielded the following results: *Lophius piscatorius*, 132 and 139 millimols per liter; *Zeus faber*, 35 millimols per liter; *Pleuronectes platessa*, 151 millimols per liter. The urine of these fish had a marked yellow color and the quantity was rather large. The steam trawl is admittedly a rough instrument; but under the circumstances it is inconceivable that the urine of the specimens of *Lophius* and *P. platessa* could have been chloride-free before catching. In the case of *Zeus faber* such a possibility could be more readily considered. In regard to *Lophius* the following data are of interest. Pitts (1934) has reported analyses on three specimens taken in an otter trawl, with urinary chloride concentrations of 2, 6, and 98 millimols per liter. Grollman (1929) has reported a chloride analysis of 56 millimols per liter on a pooled specimen of urine collected from many goosefish taken by steam trawl in the open ocean (George's Banks fishing grounds in the Atlantic). The logical interpretation of his figure is that some of the fish showed a lower and some a higher urinary chloride concentration. When the present data are taken in conjunction with those of Pitts and Grollman, it seems not unreasonable to conclude that, while the goosefish obviously has the ability to secrete a chloride-free urine, it probably can, and not infrequently does, excrete variable, and often considerable, amounts of chloride in the urine under normal conditions.

Discussion

The present observations upon urine flow and urinary chloride in marine teleosts have led to the following conclusions: (1) that freshly caught sculpins and flounders, under fairly ideal and constant experimental conditions, show a rather wide variation in the rate of urine flow and the urinary chloride concentration; (2) that there is no direct relationship between the rate of urine flow and the urinary chloride concentration. When examined in conjunction with previously recorded observations, particularly those of Pitts (1934), the present data apparently justify a third conclusion: (3) that while all marine teleosts apparently have the *capacity* to excrete a chloride-free urine, and in their normal habitat do so in the majority of instances, they not infrequently excrete variable, and at times considerable, amounts of chloride in the urine under normal physiological conditions. In addition to his data upon sculpin and goosefish, which have been discussed above and which bear out the above conclusion, Pitts presents other data upon grey sole, hake and rusty dab which lend additional support to this thesis. Obviously, the ideal material for the final solution of the problem is original bladder urine removed from large numbers of fishes of different species immediately after catching on hook and line. However, such material is exceedingly difficult to obtain. Pitts' statement that "the vast majority of fish when caught have no urine in their bladders" has been abundantly confirmed in our own experience. With respect to his data, Pitts suggests that "the relatively high per cent of fish (eleven out of a total of nineteen) [5] reported in Table I having chloride in their urine may be a result of selection for fish which, due to some condition of the normal environment, are diuretic"; and further states that "the chance of obtaining sufficient urine for analysis from a fish increases as it becomes more diuretic." *A priori*, there seems to be little basis for believing that a diuretic fish would hold his urine on catching any more consistently than a non-diuretic fish. Furthermore, the relatively large number of Pitts' freshly caught sculpins (6 out of 15) showing considerable amounts of chloride (34 to 147 millimols per liter) in their original bladder urine can hardly be accepted as diuretic (abnormal) without further proof, particularly in view of the demonstrated independence of the rate of urine flow and the urinary chloride concentration. Pitts was led to the tentative conclusion that "marine teleosts, in general, have normally a low or zero urinary chloride." All things considered, it is our belief that the alternative conclusion is more acceptable; namely, that marine teleosts normally excrete an essentially chloride-free urine in the majority of instances, but not infrequently excrete variable, and at times considerable, amounts of chloride in the urine under normal physiological conditions.

---

[5] Actually nine out of nineteen, since two of the goosefish are essentially chloride-free (2 and 6 millimols per liter).

In keeping with this latter thesis is Grafflin and Ennis's (1934) interpretation of urinary chloride as incidental to the excretion of other salts. A further implication concerns the question of a renal threshold for chloride in the marine teleosts. Marshall (1930) states: "That such a threshold for chloride exists in the case of the glomerular fish kidney is shown by examining urine from eels in salt and fresh water. In the former case the urine may contain large amounts of chloride, in the latter it rarely contains more than a trace." Yet, in the light of our present knowledge, eels in sea water almost certainly excrete an essentially chloride-free urine in the majority of instances. Furthermore, Marshall's data in the aglomerular toadfish demonstrate a a marked independence of the plasma and urinary chloride concentrations. The conclusion is drawn that the presence of a renal threshold for chloride in the marine teleosts has not been proved, and that such a threshold probably does not exist.

The situation is obviously quite complicated in the marine teleosts. We have as yet no way of determining accurately the normal rate of urine flow under conditions of existence in the sea; and there is excellent evidence that the greater part of the chloride absorbed from the gastro-intestinal tract is excreted by some extrarenal route, presumably the gills (Smith, 1930, 1932). Data obtained under fairly ideal experimental conditions have demonstrated a rather wide variation in urine flow and urinary chloride concentration, with no direct relationship between them. Furthermore, there is ample evidence to show that the presence of chloride in the urine cannot be accepted as a criterion of abnormality without further proof. From this experimental basis, the deliberate selection of animals with a low rate of urine flow (4 to 5 cc. per kilogram per 24 hours, and below) and a low or zero urinary chloride as representing the normal (see Clarke, 1934) would have to be considered as rather artificial. On the other hand, in view of the complexity of the factors involved, such a selection is undoubtedly the safest procedure to follow in physiological work until more adequate information is available.[6]

---

[6] When examined in conjunction with the present data upon urine flow and urinary chloride in the sculpin, the observations of Clarke (1934) upon xylose clearance in the same species are particularly interesting. His data indicate a rather constant mechanism of urine formation, in terms of glomerular filtration and tubular reabsorption of water (xylose U/P ratio = 4), up to a urine flow of about 10 cc. per kilogram per 24 hours.

The present study has shown that incomplete emptying of the bladder at the beginning of the urine collection period is a potential source of serious error in experimental work upon the fishes; and it is suggested that catheterization, rather than pressure alone, should be used in the preliminary emptying, and that relatively long collection periods should be used when it is practicable to do so.

The diuresis which develops in marine teleosts under the usual experimental conditions of handling has recently been seriously misinterpreted by Defrise (1934). In discussing our initial investigation of this problem (Grafflin, 1931), he speaks of the diuresis as being associated with a "continuous and progressively increasing cutaneous absorption of chloride and magnesium" (p. 697). Such a mechanism was not even implied,[7] and in subsequent writings (Smith, 1932; Pitts, 1934; Grafflin and Ennis, 1934; Clarke, 1934) there is general agreement that the salts enter the body only through the ingestion and gastro-intestinal absorption of sea water.

SUMMARY

The present observations upon urine flow and urinary chloride in marine teleosts have led to the following conclusions: (1) that freshly caught sculpins and flounders, under fairly ideal and constant experimental conditions, show a rather wide variation in the rate of urine flow and the urinary chloride concentration; (2) that there is no direct relationship between the rate of urine flow and the urinary chloride concentration. When examined in conjunction with previously recorded observations, particularly those of Pitts (1934), the present data apparently justify a third conclusion: (3) that while all marine teleosts apparently have the *capacity* to excrete a chloride-free urine, and in their normal habitat do so in the majority of instances, they not infrequently excrete variable, and at times considerable, amounts of chloride in the urine under normal physiological conditions.

LITERATURE CITED

Burian, R., 1909. Methoden zum Auffangen von Fischharn. *Zeitschr. f. Biol. Tech. u. Meth.*, **1**: 383.

Clarke, R. W., 1934. The xylose clearance of Myoxocephalus octodecimspinosus under normal and diuretic conditions. *Jour. Cell. and Comp. Physiol.*, **5**: 73.

Clarke, R. W., and H. W. Smith, 1932. Absorption and excretion of water and salts by the elasmobranch fishes. III. The use of xylose as a measure of the glomerular filtrate in Squalus acanthias. *Jour. Cell. and Comp. Physiol.*, **1**: 131.

---

[7] ". . . it seems reasonable to assume that the diuresis occurring in sculpin and toadfish with handling is dependent upon increased swallowing of sea water, brought about in some manner by the skin injury" (Grafflin, 1931, p. 609).

DEFRISE, A., 1934. Ricerche di citofisiologia renale. Il Nefrone bisegmentale. *Arch. Ital. di Anat. e di Embriol.*, **33**: 693.

EDWARDS, J. G., AND L. CONDORELLI, 1928. Studies on aglomerular and glomerular kidneys. II. Physiological. *Am. Jour. Physiol.*, **86**: 383.

GRAFFLIN, A. L., 1931. Urine flow and diuresis in marine teleosts. *Am. Jour. Physiol.*, **97**: 602.

GRAFFLIN, A. L., 1935. Chloride and total osmotic pressure in the blood of marine teleosts. *Biol. Bull.*, **69**: 245.

GRAFFLIN, A. L., AND D. ENNIS, 1934. The effect of blockage of the gastro-intestinal tract upon urine formation in a marine teleost, Myoxocephalus octodecimspinosus. *Jour. Cell. and Comp. Physiol.*, **4**: 283.

GROLLMAN, A., 1929. The urine of the goosefish (*Lophius piscatorius*): its nitrogenous constituents with special reference to the presence in it of trimethylamine oxide. *Jour. Biol. Chem.*, **81**: 267.

MARSHALL, E. K., JR., 1930. A comparison of the function of the glomerular and aglomerular kidney. *Am. Jour. Physiol.*, **94**: 1.

MARSHALL, E. K., JR., 1934. The comparative physiology of the kidney in relation to theories of renal secretion. *Physiol. Rev.*, **14**: 133.

MARSHALL, E. K., JR., AND A. L. GRAFFLIN, 1932. The function of the proximal convoluted segment of the renal tubule. *Jour. Cell. and Comp. Physiol.*, **1**: 161.

NASH, J., 1931. The number and size of glomeruli in the kidneys of fishes, with observations on the morphology of the renal tubules of fishes. *Am. Jour. Anat.*, **47**: 425.

PITTS, R. F., 1934. Urinary composition in marine fish. *Jour. Cell. and Comp. Physiol.*, **4**: 389.

SMITH, H. W., 1930. The absorption and excretion of water and salts by marine teleosts. *Am. Jour. Physiol.*, **93**: 480.

SMITH, H. W., 1932. Water regulation and its evolution in the fishes. *Quart. Rev. Biol.*, **7**: 1.

VAN SLYKE, D. D., 1923. The determination of chlorides in blood and tissues. *Jour. Biol. Chem.*, **58**: 523.

THE FORMATION OF GIANT POLAR BODIES IN CENTRIFUGED EGGS OF ILYANASSA

ANTHONY C. CLEMENT

(*From the Biological Laboratory, Princeton University*)

INTRODUCTION

The egg of the marine snail *Ilyanassa* produces a polar lobe at each of the maturation divisions and the first two cleavages. The polar lobe is normally filled with yolk and at the first cleavage passes into only one of the first two blastomeres and is thus responsible for the unequal first cleavage. Morgan (1933), in a study of the factors involved in the formation of the polar lobe, found that if eggs of *Ilyanassa* are held upside down on the centrifuge so that the normal stratification of yolk and cytoplasm is reversed, the polar lobe continues to form irrespective of the visible oöplasmic content of the region and irrespective of the position of the mitotic figure. It was observed also that if the third lobe (formed at the first cleavage) is detached from the egg, it continues to undergo changes in shape. From these results Morgan was led to infer that lobe formation ". . . Is to some extent an autonomous phenomenon, the result of conditions in the antipolar surface of the egg which is not affected by the centrifuging of the materials in its interior." In a recent series of papers Morgan (1935*a*, *b*, *c*) has extended these observations and has reported the behavior of fragments of eggs separated by centrifugal force. He finds that fragments from the animal hemisphere which contain both egg and sperm nuclei develop without any indication of lobe formation, whereas non-nucleated fragments from the vegetal hemisphere undergo rhythmic changes in shape to produce polar lobes. Unlike the normal egg, the fragments from the animal hemisphere divide into equal cells at the first and second cleavages, while at the third cleavage four micromeres are formed in normal fashion. During the summer of 1934 the author performed independently at Woods Hole a similar series of observations on animal and vegetal halves of the egg of *Ilyanassa* separated by centrifugal force. The results were essentially similar to those obtained by Morgan. Fragments consisting of the animal half of the egg were observed to develop for two or three days, giving rise to ciliated embryos capable of some slight degree of locomotion.

These observations show that the behavior of isolated animal and vegetal parts of the egg of *Ilyanassa* is, with respect to lobe formation, similar to that of corresponding parts of the egg of *Dentalium*, as described many years ago by Wilson (1904). In *Dentalium* the capacity to produce the polar lobe is restricted even before fertilization to the lower half of the egg. And if after fertilization the lower half is isolated without a nucleus, it continues to form the polar lobe as if it were still a part of the whole egg. The autonomous nature of the lobe-forming process is especially interesting in view of the important rôle which its materials play in determining development (see especially Wilson, 1904, on *Dentalium*, and Crampton, 1896, on *Ilyanassa*).

In his recent paper Morgan (1935*b*) mentions the possibility that occasional protoplasmic protrusions which he has observed in centrifuged eggs of *Ilyanassa* may be giant polar bodies, similar to those described by Conklin (1917) in centrifuged eggs of *Crepidula*. He is somewhat skeptical, however, of the validity of this interpretation. In the present paper evidence will be presented to show that giant polar bodies do form in centrifuged eggs of *Ilyanassa* and that occasionally they, instead of what would ordinarily be considered the egg, undergo development. In addition, the rather unusual stratification of egg substances under strong centrifugal force is described. Part of these observations were made at Woods Hole and part were made on eggs from snails maintained in the laboratory at Princeton. The author wishes to thank Professor E. G. Conklin for his interest in this work, and for helpful advice and criticisms concerning it.

Giant Polar Bodies

When eggs of *Ilyanassa* are suspended in a medium of equal density and centrifuged, they orient with their yolk-laden vegetal poles outward. The egg substances are easily stratified into three zones: oil at the animal pole, yolk at the vegetal pole, and granular cytoplasm in between. Nuclei come to lie in the cytoplasmic zone. Maturation spindles, at and after the metaphase stage, are anchored to the periphery at the animal pole and hence offer resistance to displacement. If, however, the eggs are so oriented on the centrifuge that the point of attachment of the maturation spindle lies well within the region where the oil cap is formed, the incrowding of the oil droplets may force the denser spindle away from its point of attachment. The spindle may thus come to lie wholly or partly in the cytoplasmic zone. In such cases, strands of denser cytoplasm may be seen, in fixed and stained preparations, connecting one end of the maturation spindle with the periphery at the animal pole (Fig. 14). The cell wall at the point of attachment of these strands may be indented by the pull exerted on it during centrifuging.

Whether normal or abnormal polar bodies form in such centrifuged eggs depends on the position of the maturation spindle when the division takes place. If the spindle returns to its original position at the animal pole, a normal polar body may be formed. If it does not return before division sets in a giant polar body is formed. Smaller "giants" may contain only a part of the oil cap (Fig. 18), larger ones may contain all of the oil cap (Fig. 16), and still larger ones may contain a part of the cytoplasmic zone (Fig. 17). In experiments to be described in this paper the eggs were centrifuged after the formation of the first polar body and before the formation of the second. The position of the first polar body thus served as a convenient marker of the animal pole. When giant polar bodies were formed they resulted from the second maturation division. The fate of such giant polar bodies varied. In some cases the giant polar body underwent no further development, while the egg proper developed normally. In other cases the egg proper did not cleave, although it did undergo rhythmic contractions to form the polar lobe, while the giant polar body underwent development. Whether the giant polar body or the egg proper is to develop seems, without doubt, to depend on the presence or absence of the sperm nucleus. In eggs centrifuged in the interval between the maturation divisions, or earlier, the condensed sperm nucleus comes to lie in the cytoplasmic zone, near the boundary between the latter and the oil zone (Figs. 14, 15, 23). In cases where the giant polar body includes a portion of the cytoplasmic zone, it would sometimes include also the sperm nucleus. The giant polar body would then be fertilized by the sperm nucleus and would therefore develop, while the part that would ordinarily be considered the egg, although containing the female pronucleus, would be unfertilized and hence would not develop.

Experiments

The data from three experiments will be summarised to illustrate the production of giant polar bodies. In all cases the eggs were suspended in a gum arabic-sea water solution and rotated on an electric centrifuge equipped with hæmatocrit head.

1. Beginning 17 minutes before the second polar body was due, eggs were centrifuged at a moderate speed for 7 minutes. Three zones were separated and the eggs slightly elongated (Fig. 1). Thirty minutes after the eggs were removed from the centrifuge a constriction between the cytoplasmic and oil zones announced the beginning of the

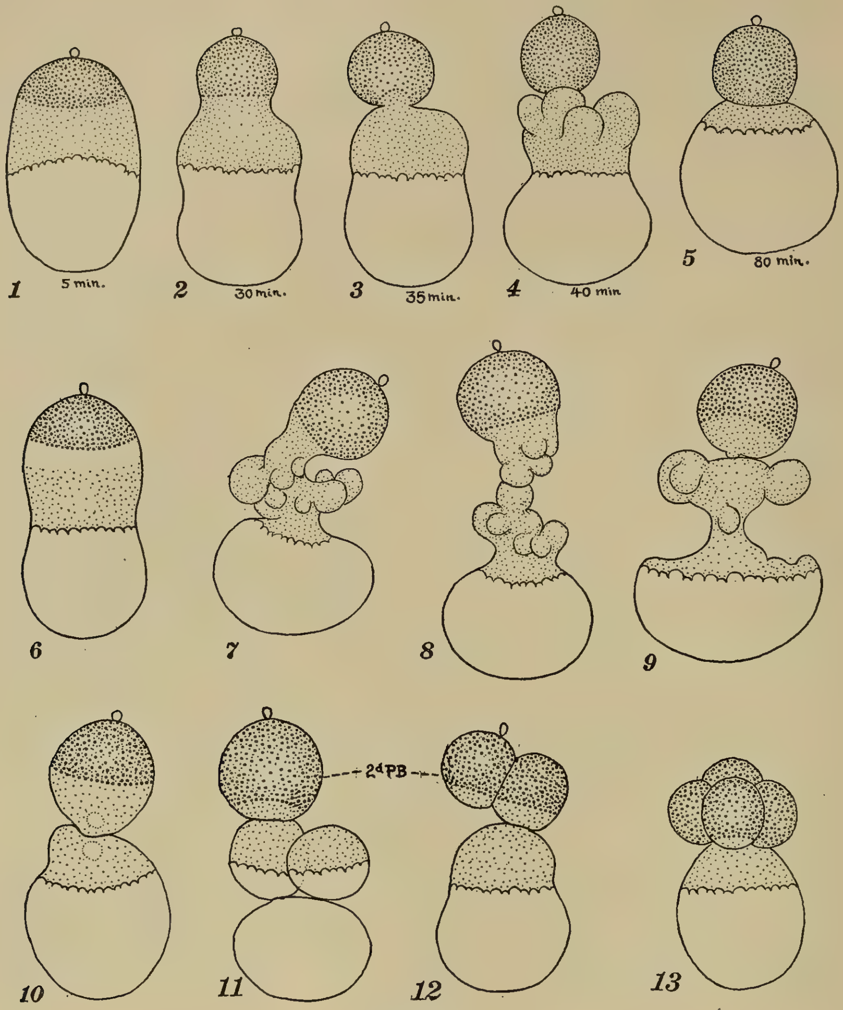


Plate I

Figs. 1–5. Same egg at successive intervals after centrifuging, showing formation of giant second polar body. See Experiment 1 of text. 1, 5 minutes; 2, 30 minutes; 3, 35 minutes; 4, 40 minutes; 5, 80 minutes.

Figs. 6–13. Showing behavior of eggs centrifuged in interval between maturation divisions. See Experiment 2 of text.

6. Egg 5 minutes after centrifuging.

7–9. Three different eggs 40–45 minutes after centrifuging. Second maturation division producing giant polar bodies. Note cytoplasmic lobes.

10. Egg 70 minutes after centrifuging; giant second polar body.

11. Egg 2½ hrs. after centrifuging. Trefoil stage of first cleavage; polar body undivided.

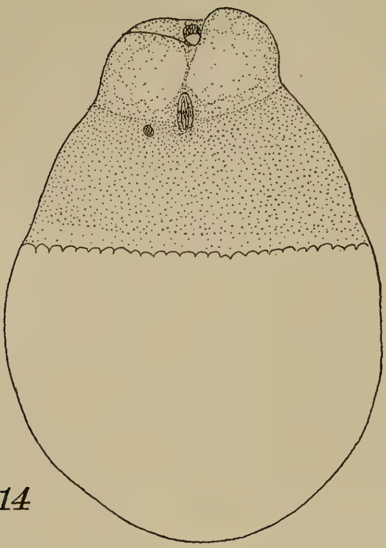
12. Another egg 2½ hrs. after centrifuging. Egg undivided; giant polar body cleaved.

13. Egg 3½ hrs. after centrifuging. Giant polar body in 4-cell stage.

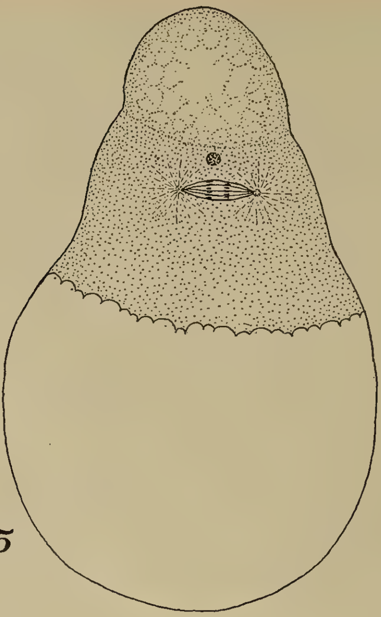
second maturation division; a constriction in the yolk zone represented formation of the polar lobe (Fig. 2). Ten minutes later the oil zone was constricted off as a giant second polar body and around the animal end of the egg a cluster of cytoplasmic lobes had appeared (Fig. 4). Six minutes later the cytoplasmic lobes had been resorbed. The polar lobe constriction persisted for some minutes longer, but eventually disappeared (Fig. 5). An hour after the giant polar bodies were formed a part of the eggs were fixed and stained. Two of them are shown in Figs. 16 and 17. The first polar body is normal, the second is large and contains the oil cap (Fig. 16) or oil cap plus part of the cytoplasmic zone (Fig. 17), as well as chromatin from the second maturation division. Two hours and forty minutes after the giant second polar bodies were formed the first cleavage had occurred; the eggs were fixed and stained. In some cases the egg showed normal first cleavage (Fig. 19); the nuclear membrane in the giant polar body had dissolved and the chromatin was lying free in the cytoplasm. In other cases the egg proper showed a polar lobe constriction but no cleavage, whereas the giant second polar body had cleaved (Fig. 20). The two small cells resulting from the division of the giant polar body contained normal looking nuclei; the egg proper contained chromosomal vesicles from the second maturation division.

2. Beginning 12 minutes before the second polar body was due another lot of eggs was subjected at alternate 5-minute intervals to centrifuging and rest over a 30-minute period (Fig. 6). Forty minutes after the eggs were removed from the centrifuge and one hour after the second polar body was given off in the controls, the second maturation division occurred. The division plane cut through the cytoplasmic zone, thus giving rise to giant polar bodies. At the time when the division was just being completed, the cytoplasm of both giant polar body and egg proper protruded numerous lobes. These persisted for several minutes and were then resorbed (Figs. 7–10). One hour and forty minutes after the giant second polar body was formed, the first cleavage had occurred. In some cases the egg proper cleaved and the giant polar body underwent no change (Fig. 11), while in other cases the egg proper remained uncleaved and the giant polar body cleaved (Fig. 12). One hour later the giant polar body was in the four-cell stage (Fig. 13); a fixed and stained preparation is shown in Fig. 21.

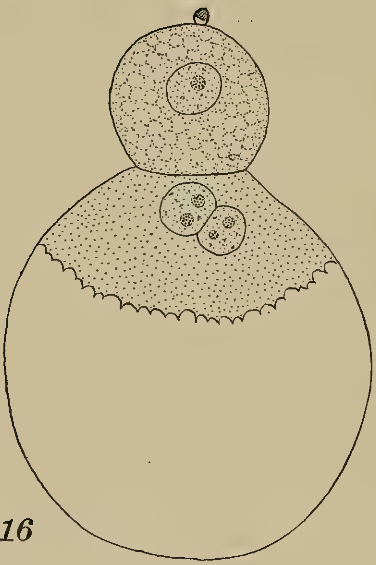
3. In this experiment the eggs were broken into animal and vegetal halves. Eggs with the first polar body off were centrifuged slowly for 25 minutes (11:13–11:38 A.M.); they were greatly drawn out in the middle so that only long, slender necks of protoplasm connected the animal and vegetal halves. By agitation with a stream of water from



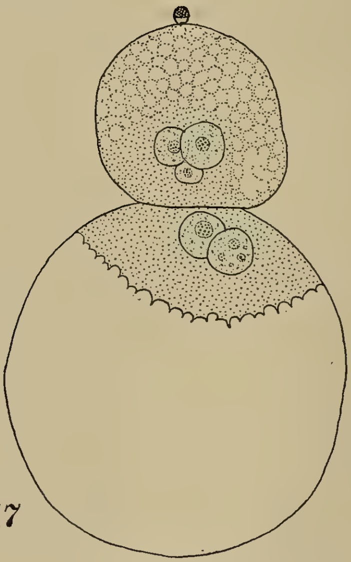
14



15



16



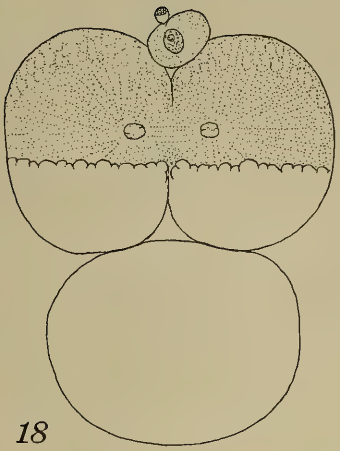
17

PLATE II

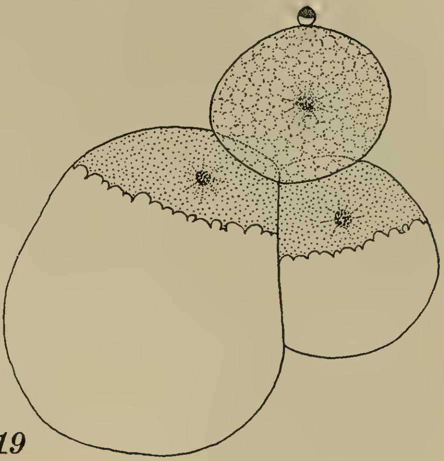
FIG. 14. Egg centrifuged for 5 minutes. Second maturation spindle forced away from animal pole but remains connected by strands of cytoplasm. Condensed sperm nucleus at border of oil and cytoplasmic zones.

FIG. 15. Egg centrifuged intermittently over 30-minute interval (See Experiment 2 of text), then fixed immediately. First polar body lost. Second maturation spindle lying free in cytoplasmic zone. Sperm nucleus between spindle and oil zone.

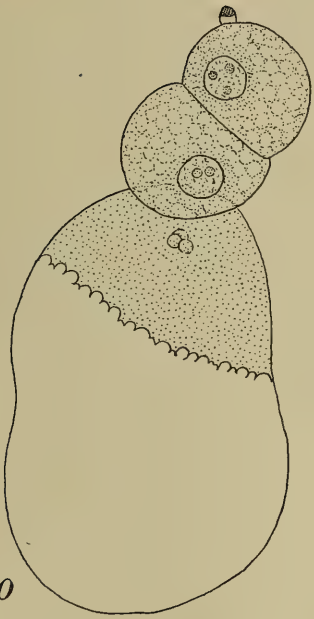
FIGS. 16, 17. Giant polar bodies in eggs centrifuged before second maturation division (See Experiment 1 of text).



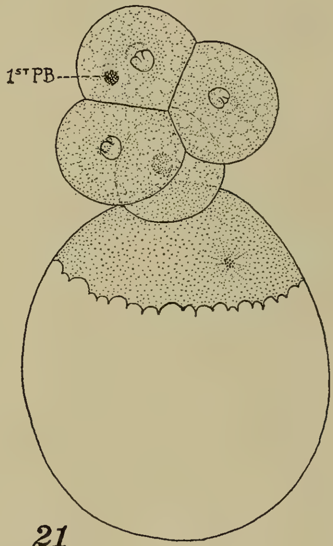
18



19



20



21

PLATE III

Eggs from different lots centrifuged in interval between maturation divisions and preserved later.

FIG. 18. Second polar body larger than normal; egg in trefoil stage of first cleavage.

FIG. 19. Giant second polar body contains all of oil zone. First cleavage of egg normal.

FIG. 20. Giant second polar body has divided; egg undivided.

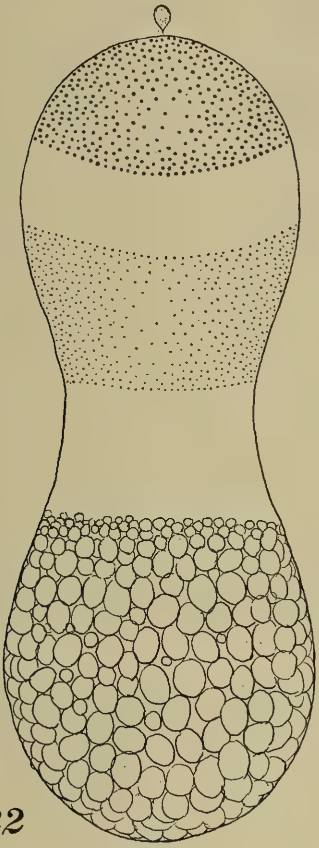
FIG. 21. Egg undivided, giant second polar body in 4-cell stage.

a fine pipette the animal and vegetal halves were separated. At 12:15 P.M. the vegetal halves showed polar lobe constrictions and the animal halves were beginning to divide. By 12:20 the animal halves had divided equatorially into two equal cells (second maturation division); both parts showed, during the succeeding minutes, small cytoplasmic lobes, as described in the preceding experiments. At 12:30 the two cells of the animal half were round, and likewise the isolated vegetal halves were round. At 1:25 the vegetal halves were again constricted, and the polar moiety (giant polar body) of the animal halves cleaved into two cells. The vegetal halves now became spherical again, but at 3:30 showed another constriction; at this time the giant polar body underwent a second cleavage. Obviously, in this experiment the sperm nucleus was included in the giant second polar body; the central cytoplasmic part contained chromatin from the second maturation division, but no sperm nucleus.

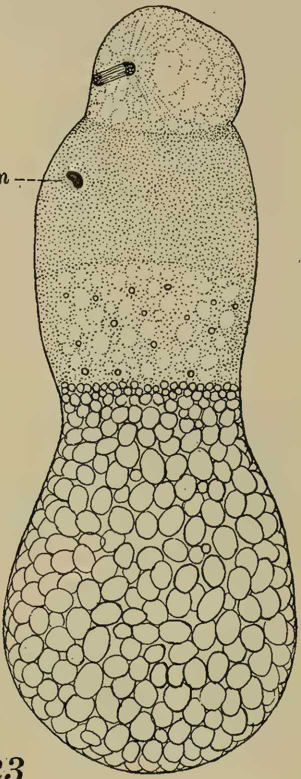
The Zones of the Centrifuged Egg

With moderate centrifuging the contents of the egg of *Ilyanassa* are divided into three zones. Since the eggs orient on the centrifuge, the yolk particles are merely concentrated in the vegetal half; oil drops accumulate as a cap around the animal pole; the cytoplasmic zone which lies between these two contains many fine, colorless granules, as well as scattered oil droplets and smaller yolk particles. If eggs are subjected to harder and more prolonged centrifuging, further separation of substances takes place. First, the fine granules of the middle zone are thrown down so that a hyaline zone appears beneath the oil cap. At this stage the eggs may pull into halves. In other cases further stratification takes place. A second clear band appears in the cytoplasm just above the yolk zone. Five zones are now present: (1) oil cap, (2) upper clear zone, (3) fine, colorless granules, (4) lower clear zone, and (5) yolk (Fig. 22). This stratification has been observed in fertilized eggs of various stages and in unfertilized eggs (obtained by tearing apart the body of the female) in which the germinal vesicle was still intact. Eggs are more resistant to stratification before the germinal vesicle has dissolved than afterwards. A similar decrease in cytoplasmic viscosity upon the breakdown of the germinal vesicle has been noted by many observers in a variety of other eggs. The germinal vesicle in the centrifuged *Ilyanassa* egg comes to lie beneath the oil cap. Within the germinal vesicle chromatin and the large nucleolus are thrown to the centrifugal end.

The nature of the five zones of the fully stratified egg will now be considered. The lightest and heaviest zones consist, respectively, of oil droplets and yolk particles. The upper clear zone consists of hyaline protoplasm which in living eggs appears homogeneous and structureless. The fine, colorless granules of the central band stain readily with Janus Green B. This is best observed by placing eggs in a weak solution of the dye for a few minutes before centrifuging. Upon centrifuging, the colored band of granules then stands out quite clearly. The size and relative density of these granules and their affinity for Janus Green B indicate that they are mitochondria.



22



23

Plate IV

Fig. 22. Living egg, centrifuged for 19 minutes. Five zones of materials separated: oil; upper clear zone; fine, colorless granules; lower clear zone; yolk.

Fig. 23. Fixed and stained preparation of whole egg, centrifuged during anaphase of first maturation division; upper clear zone granular, lower clear zone vacuolated.

However, in sections of centrifuged eggs prepared according to the Altmann technic for mitochondria, these granules showed no selective staining, but the yolk particles instead took up acid fuchsin. In spite of this discrepancy, it seems likely that they are comparable to the fine, colorless granules of other eggs, notably those of the sea urchin egg (Wilson, 1926; Harvey, 1933), which have been identified as mitochondria. The lower or heavier clear zone is quite different from the upper one. In centrifuged eggs which had been killed in formalin, treated with Sudan III and cleared in glycerin, the upper clear zone appeared finely granular, while the lower clear zone showed a meshwork of faintly visible cytoplasmic strands amongst which could be seen lacunæ or alveoli and a few small yolk spherules. In centrifuged eggs fixed in Kleinenberg's picro-sulfuric fluid and stained in Conklin's modification of Delafield's hæmatoxylin, the upper zone stains freely and appears densely granular, whereas the lower zone stains faintly, is not so granular, and has a faintly but definitely vacuolated appearance (Fig. 23).

Such a stratification as this may mean either (1) that under centrifugal force a gradient of density is established in the clear cytoplasm and that the fine granules possess a specific gravity intermediate between that of the heavier and lighter cytoplasms, or (2) that the fine granules are thrown down against a preformed region of gelated or partially gelated hyaline protoplasm and that they pile up against this zone to form a band. The latter interpretation seems to be the more likely one, especially in view of the difficulty of displacing yolk particles from this region.

DISCUSSION

The formation of giant polar bodies in centrifuged eggs of *Ilyanassa*, as described in this paper, is essentially similar to that observed by Conklin (1917) in eggs of *Crepidula* centrifuged during the maturation period. For greater cytological details and a comprehensive review of the subject, the reader is referred to his paper. Centrifuging *Crepidula* eggs in sea water, Conklin found that by mutual pressure the eggs were prevented from orienting in the tubes so that in many cases yolk was thrown to the animal pole. Thus maturation spindles could be forced far away from their normal positions. Polar bodies of varying size and composition could be given off at any position on the egg surface, even at the vegetal pole. Displaced nuclei and cytoplasm in time returned to the animal pole, however, so that the polarity of the egg remained unchanged. Giant polar bodies did not develop because they did not receive a spermatozoön and they did not receive a spermatozoön because they formed after the egg had been fertilized and the cortical layer rendered impervious to further sperm entry. In some cases, however, the daughter cell of the second maturation division which lay at the animal pole and which in this paper has been referred to as the giant polar body, received the sperm nucleus and hence developed. That part of the egg which developed Conklin referred to as the egg, whereas the other part, whether at the animal or vegetal pole, he referred to as the giant polar body.

The peculiar activity of the egg which results in the production of clusters of cytoplasmic lobes at the close of the second maturation division is reminiscent of similar cytoplasmic activity in dividing tissue-culture cells, as demonstrated by Canti and by the Lewises in motion-pictures. Somewhat similar lobe production at the time of the maturation divisions has also been observed by Morgan and Tyler (1935) in centrifuged eggs of *Urechis*. The normal egg of *Ilyanassa*, it will be remembered, produces a polar lobe at the time of the maturation divisions. The cluster of cytoplasmic lobes around the region of the fading maturation figure in centrifuged eggs is another phenomenon, however, as is evident from the behavior of isolated animal and vegetal halves of the egg: the isolated vegetal half continues to form the polar lobe at the usual times, while the nucleated animal half does not form the polar lobe, but does produce the cluster of cytoplasmic lobes. These cytoplasmic lobes evidently result from some change in the egg accompanying the division process. They have not, however, been observed in the normal egg. It may be that they appear in the centrifuged and elongated egg because the cell wall and cortex have been weakened by undue stretching.

The usual stratification of substances in strongly centrifuged eggs includes a layer of oil droplets at the centripetal pole, two or three layers of granules (mitochondria, yolk, pigment, etc.) at the centrifugal pole, and an intermediate zone of hyaline protoplasm. Some exceptions are known. In *Tubifex*, according to Parseval (1922), the yolk occupies an intermediate position between two clear zones. In *Sphærechinus granularis*, Ethel B. Harvey (1933) has observed two zones of clear protoplasm, one in the usual position next to the oil cap and the other at the centrifugal pole. *Ilyanassa* differs from the usual situation in that two clear layers are present and separated by a band of fine granules. As has been pointed out, this evidently means either (1) that a gradient of density is established in the clear cytoplasm, or (2) that the more centrifugal clear zone represents a region so firm in consistency that the fine granules cannot penetrate it. In this latter respect it may resemble the central spongy area of the egg of *Chætopterus*, as described by Lillie (1909), which by virtue of its resistant wall neither allows granules from other regions to pass through it nor allows those granules within it to pass out of it, except with very strong centrifugal force.

SUMMARY

1. If the second maturation spindle of the egg of *Ilyanassa* is displaced from the animal pole by centrifugal force, a giant polar body may be formed. If the sperm nucleus is included in the giant polar body the latter, instead of the egg proper, may develop.

2. The close of the maturation division in centrifuged and elongated eggs is attended by the production of clusters of cytoplasmic lobes around the region where the achromatic figure lay. These lobes are distinct from the normal polar lobe.

3. In strongly centrifuged eggs five zones of substances are separated. These include two clear zones separated by a band of fine, colorless granules.

BIBLIOGRAPHY

CONKLIN, E. G., 1917. Effects of centrifugal force on the structure and development of the eggs of Crepidula. *Jour. Exper. Zoöl.*, **22**: 311.

CRAMPTON, H. E., 1896. Experimental studies on gasteropod development. *Arch. f. Entw.-mech.*, **3**: 1.

HARVEY, E. B., 1933. Development of the parts of sea urchin eggs separated by centrifugal force. *Biol. Bull.*, **64**: 125.

LILLIE, F. R., 1909. Polarity and bilaterality of the annelid egg. Experiments with centrifugal force. *Biol. Bull.*, **16**: 54.

MORGAN, T. H., 1933. The formation of the antipolar lobe in Ilyanassa. *Jour. Exper. Zoöl.*, **64**: 433.

MORGAN, T. H., 1935*a*. Centrifuging the eggs of Ilyanassa in reverse. *Biol. Bull.*, **68**: 268.

MORGAN, T. H., 1935*b*. The separation of the egg of Ilyanassa into two parts by centrifuging. *Biol. Bull.*, **68**: 280.

MORGAN, T. H., 1935*c*. The rhythmic changes in form of the isolated antipolar lobe of Ilyanassa. *Biol. Bull.*, **68**: 296.

MORGAN, T. H. AND ALBERT TYLER, 1935. Effects of centrifuging eggs of Urechis before and after fertilization. *Jour. Exper. Zoöl.*, **70**: 301.

VON PARSEVAL, M., 1922. Die Entwicklung zentrifugierter Eier von Tubifex rivulorum Lam. *Arch. f. Entw.-mech.*, **50**: 468.

WILSON, E. B., 1904. Experimental studies on germinal localization. I. The germ-regions in the egg of Dentalium. *Jour. Exper. Zoöl.*, **1**: 1.

WILSON, E. B., 1926. Newer aspects of the alveolar structure of protoplasm. *Am. Nat.*, **60**: 105.

THE EFFECT OF TEMPERATURE ON THE RATE OF THE FERTILIZATION REACTION IN VARIOUS MARINE OVA

H. Y. CHASE[1]

(*From the Hopkins Marine Station, Stanford University, California*)

Introduction

The development of a fertilization membrane in most marine ova serves as an indicator of the fertilization reaction. The process is important because of its relation to the cortical reaction of the egg and its conspicuous part in it. Loeb (1906, 1910, 1916) and Loeb and Wasteneys (1915) have presented a large body of evidence supporting the view that the essential feature of fertilization is the increase in the rate of oxidations and that this increase is caused by membrane formation, or more strictly, by the reactions normally resulting in the development of the fertilization membrane. More recently Chase (1935) has shown that the fertilization membrane develops in some eggs by a radical transformation of the vitelline membrane, in others chiefly by the separation of a tough pre-existing membrane from the surface of the egg. Loeb's work was done mainly on the sea urchin egg, which belongs in the former category. It is not improbable that the development of the fertilization membrane in part may be a different type of process in eggs which have a tough membrane before activation.

This paper reports a study of the effect of temperature on the development of the fertilization membrane in several kinds of eggs. Measurements on the effect of temperature fall in the following categories: (*A*) effect on gametes of exposure to various temperatures, (*B*) the effect of temperature on the time until contact of the sperm with the egg, and (*C*) the effect of temperature on the rate of the development of the fertilization membrane.

Material and Methods

The experiments were performed during the spring and summer of 1933 and the winter and spring of 1934 at the Hopkins Marine Station, Pacific Grove, California.[2] Eggs of the following species were used:

---

[1] This work was done during the tenure of a fellowship from Howard University, made available by a grant from the General Education Board.

[2] The writer wishes to express his gratitude to Dr. D. M. Whitaker, Department of Zoölogy, Stanford University, who directed the work. He is also indebted to Dr. W. K. Fisher and the staff at Hopkins Marine Station for their many courtesies.

the sand dollar, *Dendraster excentricus;* the sea urchin, *Strongylocentrotus purpuratus;* the starfish, *Patiria* (*Asterina*) *miniata;* and the echiuroid, *Urechis caupo*, Fisher and MacGinitie (1928*a*, 1928*b*), These forms are found in abundance in the vicinity of the station.

Animals (except *Urechis*) were collected each day when an experiment was made in order to insure that mature gametes in the best condition were used. Sand dollars were dredged from Monterey Bay and sea urchins and starfish were collected at low tide from pools where the water was fairly deep. The large worm, *Urechis*, which lives in U-shaped burrows in mud flats of estuaries along the California Coast, was dug at Elkhorn Slough, about 20 miles from the station, when the tides were favorable.

Gametes of the echinids were obtained by cutting out the gonads of the animals. Bits of ovary were placed in finger bowls containing sea water and removed after the eggs had exuded. Portions of testis were placed in clean dry Syracuse watch glasses where the sperm were shed. Starfish were induced to spawn when they were brought into the laboratory and spread on wet sea weed, and the gametes were pipetted off as they were extruded. The eggs were placed in finger bowls containing sea water and the sperm were placed in Syracuse watch glasses. Eggs and sperm of *Urechis* were collected by means of a small bore pipette inserted into the gonopore of an animal. The eggs were kept in finger bowls containing sea water and the sperm were placed in Syracuse watch glasses.

Standard sperm suspensions were made as they were needed by adding a drop of dry sperm to 5 cc. of sea water. Every precaution was taken during the handling of the eggs to prevent premature activation. Samples from each finger bowl were observed frequently and when eggs with fertilization membranes were found the whole dish was rejected.

For regulation of temperature a water bath with a transparent bottom was constructed and fitted to the microscope. A flat-bottomed glass vessel, the fertilization chamber which contained sea water, was placed within the bath. A glass slide upon which the gametes were placed was suspended within this chamber so that it was below the surface of the sea water and within the focus of the microscope. The bulb of a calibrated thermometer rested on the slide adjacent to the gametes. Any desired temperature down to 2° C. could be maintained constant to within ± 0.25° C.

All observations of eggs and sperm were made at a magnification of 180 diameters.

THE EXPERIMENTS

Since it is generally known that the development of many marine eggs is slower at low temperatures than at higher temperatures, it is important to know to what extent changes in temperature affect those initial reactions which underlie the developmental process. The present section details experimental results concerning the effect of temperature on the rate of the cortical reaction.

Effect on Gametes of Exposure to Various Temperatures

Gametes were subjected to a range of temperature from 2° C. to the highest viable temperatures. Observations were made on the activity of sperm at extreme temperatures as compared with the activity of sperm in a control run at 15° C. (the approximate average temperature of water in Monterey Bay). After temperature adjustment a drop of fresh sperm suspension was placed in the fertilization chamber and observed microscopically. At 2° C. sperm of *Dendraster* and *Patiria* became feebly active while sperm of *Strongylocentrotus* and *Urechis* were very active. At temperatures of 4° C.–10° C. sperm of all species showed increased activity with rise in temperature. When sperm of each species were observed at 25° C. they were found to be very active but at 28.5° C.–30° C. sperm of *Dendraster* and *Patiria* became inactive. The sperm of *Strongylocentrotus* and *Urechis* were slightly more resistant to the higher temperatures. They became inactive between 35° C. and 40° C.

Unfertilized eggs were observed at extreme temperatures. At 2° C. eggs of *Dendraster*, *Strongylocentrotus*, and *Urechis* were apparently not affected by cold. However, starfish eggs were activated by very low temperatures as was shown by Greeley (1902). Eggs of *Patiria* were affected by temperatures between 2° C. and 7° C. Immediately after the eggs were dropped into the sea water at 2° C.–4° C. large blisters formed in the outer layer of each egg and the cytoplasm receded from the egg membrane. This phenomenon was followed by the abnormal cleavage of the egg in some cases and in most cases by fragmentation. At slightly higher temperatures, 5° C.–7° C., smaller blisters formed and the egg membrane began to separate from the underlying cytoplasm which in a few cases gave a fairly regular cleavage, while in many other cases the egg fragmented. A few eggs were activated by temperatures as high as 8° C.–8.5° C. while others were more resistant to cold and were not activated at temperatures as low as 6° C. At 29° C. and above eggs were affected by heat. Eggs of *Dendraster* usually formed one or more large blisters and at 32° C.

they cytolyzed. Eggs of *Strongylocentrotus* formed blisters at 28° C.–32° C. and cytolyzed above 32° C. When eggs of *Patiria* were exposed to a temperature of 30° C. membrane separation began, followed by cytolysis. Eggs of *Urechis* were more resistant to heat than eggs of the other species, showing the beginning of the separation of membranes at 35° C. and cytolyzing at higher temperatures.

The Effect of Temperature on the Time until Contact of the Sperm with the Egg

A drop of adequately concentrated sperm suspension was placed in the fertilization chamber which contained sea water at a particular temperature. A small drop containing eggs was then placed in the chamber and the time of insemination was recorded by stop watch the instant that the eggs came into the sperm sea water. The time of sperm contact was recorded when the first sperm was seen at the surface of an egg. In each case a control was run at 15° C., and the time for contact at this temperature was compared with that at the other experimental temperatures. All eggs studied except those of *Urechis* were enclosed within a relatively thick layer of jelly. In the case of the latter the jelly was only about 5 to 10 microns thick.

The duration of the appreciable time-lapse depended principally upon the activity of the sperm at the various temperatures, but perhaps also to some extent upon the physical consistency of the jelly. During most of the time-lapse the sperm were swimming through the layer of jelly. This took longer at low temperatures. Numerous measurements were made, usually 10 to 20 or more with each species at each temperature. The final results were obtained by averaging the time between insemination, and the contact of the fastest swimming sperm with the egg, rather than averaging all of the measurements in each experiment. The data are given in Table I.

The Effect of Temperature on the Rate of Development of the Fertilization Membrane

The effect of temperature on the rate of the reactions leading to the development of the fertilization membrane was determined by inseminating eggs in the manner described in the preceding section at temperatures ranging from 2° C. to the highest temperature at which normal fertilization took place. No eggs were used unless 95 to 100 per cent of a sample developed fertilization membranes at 15° C.

In each experiment about ten eggs came to lie in the field of the microscope. Time was measured from the instant the eggs were dropped into the sperm suspension, until the beginning of the development of the fertilization membrane was observed on that egg which represented the reaction of half of the eggs in the field of the microscope (usually the fifth egg to react).

The actual reaction-time (the time-lapse from contact of the spermatozoön with the egg to the beginning of the development of the fertilization membrane) was determined by subtracting the average values of the time required for the sperm to pass through the egg jelly at a given temperature (Table I) from the time measured from insemination to the beginning of the development of the fertilization membrane. The data for the actual reaction-time are shown graphically in Fig. 1.

TABLE I

Approximate minimum time (seconds) for sperm to reach the egg surface at different temperatures.

Temperature	Dendraster	Strongylocentrotus	Patiria	Urechis
°C.				
2	41.3	9.3	—	10.3
3	39.1	7.1	—	10.0
4	34.1	5.6	30.4	9.6
5	28.9	5.1	25.2	8.9
7	23.0	4.3	23.9	6.7
10	17.8	3.3	17.3	3.7
12	12.0 *	2.9 *	14.0	2.6
15	5.3	2.6	13.4	1.6
17	5.0 *	2.2 *	11.3	1.8
20	4.5	1.9	9.9	1.0
22	7.5	1.9	9.1	0.9
25	8.0	2.1	7.9	0.9
27	8.9	2.2 *	6.9	1.8
27.5	9.2	—	—	—
28	9.2	—	6.1	—
28.5	13.4	—	6.6	—
29.5	—	—	5.9	—
30	—	2.4	—	3.1
31	—	2.5 *	—	—
31.5	—	2.6	—	—
32	—	2.7	—	3.8
35	—	—	—	3.8

* Interpolated values.

DISCUSSION

A study of the effect of temperature on the rate of the cortical reactions which lead to the development of the fertilization membrane gives objective information concerning the most important parts of the process, namely, the invisible reactions which underlie membrane development and which presumably initiate the development of the egg. The fertilization membrane is of little developmental importance once it is fully formed; its development being merely the indicator of

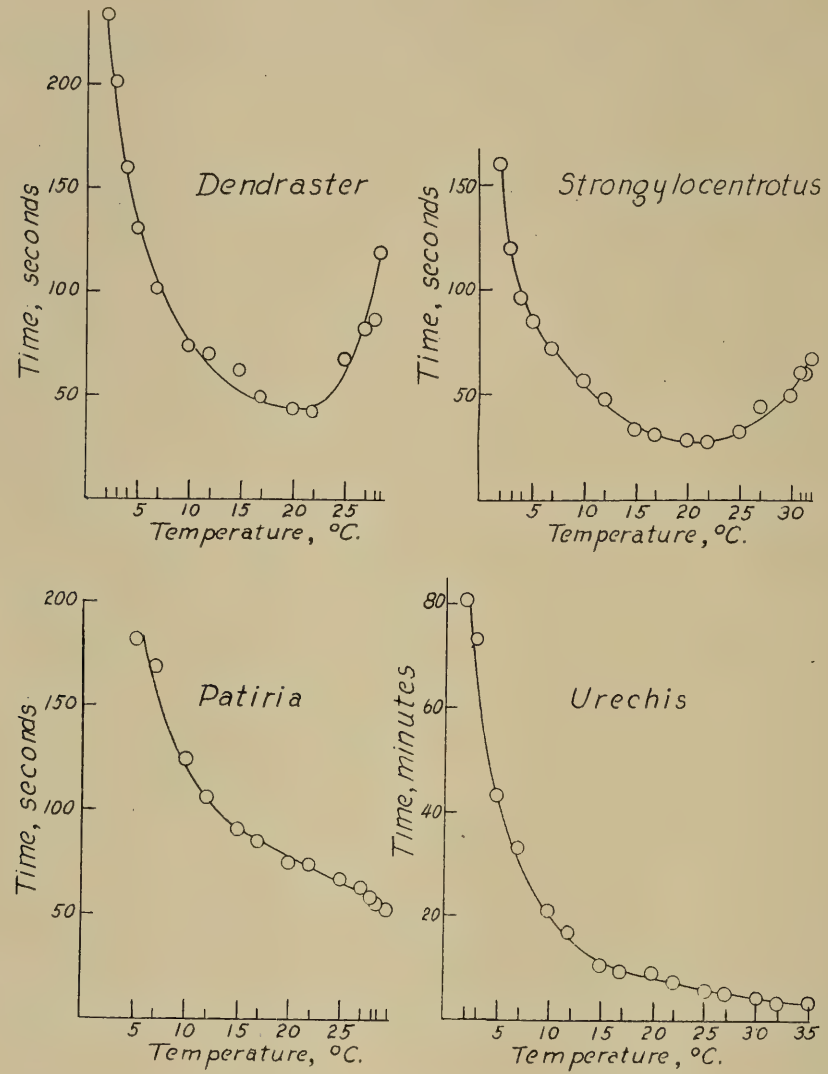


FIG. 1. Effect of temperature on the time of the beginning of the development of the fertilization membrane after contact of the sperm with the egg. Each circle represents the average of 20 to 95 measurements (average number of measurements = 43.8), each on about ten eggs.

the underlying cortical reaction. It is of interest in this connection, especially, to know when and how the development of the fertilization membrane takes place.

Explicit Definition of the Phenomenon Measured

In measuring the time-lapse from the contact of the sperm with the surface of the egg to the beginning of the development of the fertilization membrane, it must be pointed out that in none of the eggs studied does the development of the membrane begin at all points of the egg surface at the same time. It starts at one point, usually the entrance point of the sperm. After a brief time-lapse, which is greater the greater the distance from this initial point, the membrane starts to develop at all other points. Since the sperm may enter the eggs of the four species at any point, and presumably with equal probability, oftener than not the first point at which the development of the fertilization membrane begins is not in the focal plane of the microscope. In about half of the cases this point is on the under side of the egg and is not visible. The practicable locus of observation where accurate determinations can be made is the geometrical equator of the egg as seen from above, and this is the focal plane of the microscope and the place where the beginning of the development of the fertilization membrane was timed.

If the sperm entered at the equator, the observed time for the beginning of the development of the membrane would be the minimum for the whole egg. The other limit would be the entrance of the sperm at a point 90° above or below the optical equator of the egg. In this case the beginning of membrane development at the equator would be at a point one-fourth the circumference of the egg removed from the entrance point of the sperm. Since the entrance point and the resting position of the observed egg bear chance relationship, the average of the measurements would represent the time-lapse from the contact of the sperm with the egg surface to the beginning of the development of the fertilization membrane at a point one-eighth of the circumference of the egg from the entrance point of the sperm. This is the time-lapse measured in the eggs of the four species, and since each average includes hundreds of eggs, a good average has been obtained.

Temperature Coefficients

Since chemical reactions usually increase in rate with rise in temperature in such a way that the rate is approximately doubled when the temperature is increased 10° C. (Q_{10} = approximately 2.0), the order of magnitude of Q_{10} values has been used extensively in biological work to determine whether the rate of a measurable process is limited by the rate of metabolic or chemical reaction on the one hand, or by physical factors (Snyder, 1908). Loeb and Wasteneys (1911) have shown that the Q_{10} values over the viable range for the rate of oxygen consumption by *Arbacia* eggs is approximately constant and approximately equal to 2.0. On the other hand, the temperature coefficients for the rate of cell division in this same form were shown to range from $Q_{10} = 7.3$ (7° C.–17° C.) to $Q_{10} = 1.7$ (20° C.–30° C.). The rate of cell division in the lower temperature ranges is evidently limited by factors other than metabolic rate, or as Loeb and Wasteneys conclude, oxidation is not the independent variable in development. In the middle temperature ranges, however, the temperature coefficient is that of a chemical reaction. Fauré-Fremiet (1913) finds that the Q_{10} values for cleavage rate in *Ascaris* eggs increases from -4.1 between 37° C. and 42° C. to 6.25 over the range from 6° C. to 16° C. He also concludes that factors other than chemical reactions enter into limiting the rate of cell division and he suggests viscosity as another factor, especially since he had previously shown in *Ascaris* that the viscosity had a Q_{10} value of about 5.0 to 5.8.

Ephrussi (1927) has accurately determined the temperature coefficients (Q_{10}) of various stages in mitosis in eggs of *Paracentrotus* and *Ascaris*. He found that the values agreed very closely indeed in the two forms at corresponding stages. The coefficients determined in the middle ranges of the viable temperature ranges for the forms used (18.5° C.–26° C. for *Paracentrotus;* 24° C.–34° C. for *Ascaris*) indicate that the process is chiefly physical. For example, the change in the state of nuclear material after the end of the anaphases in *Paracentrotus* shows a coefficient of $Q_{10} = 1.0$. For swelling or imbibition of the sperm nucleus $Q_{10} = 1.6$.

Snyder (1911) points out that in chemical reactions Q_{10} is not strictly constant. Bayliss (1924, p. 42) emphasizes the variability of temperature coefficients in various chemical systems and over different temperature ranges. It must be kept in mind then that temperature coefficients may be used only as a somewhat rough indication of reaction type, and that it is the order of magnitude of the Q_{10} values, rather than very exact figures, which is significant. For the comparatively simple purpose of the treatment to follow, the Van't Hoff coefficient (Q_{10}) is used rather than the temperature characteristic (μ) of the Arrhenius equation, which has been used especially by Crozier (1924) for more refined analysis of temperature effects.

The Temperature Coefficients of the Reactions Leading to the Development of the Fertilization Membrane.—Temperature coefficients (Q_{10}) were calculated from the data in Fig. 1 after Snyder (1908) as follows:

$$Q_{10} = \left(\frac{K_2}{K_1}\right) \frac{10}{t_1 - t_2},$$

where K_2 is the time-lapse at the lower temperature t_2 and K_1 is the time-lapse at the higher temperature t_1. When the reaction rate diminishes with rise in temperature the fraction, K_2/K_1, is inverted and a negative sign is given the coefficient.

The calculated values of Q_{10} for eggs of the four species studied are given in Table II. An inspection of the table shows at once that the temperature coefficients are not constant throughout the temperature ranges of fertilization nor are they of the order of magnitude characteristic of chemical reactions except in the middle temperature ranges. As temperature decreases there is an increase from values of the order of 1.3 or 1.5 to values altogether above the range characteristic of metabolic processes, except in *Patiria* where the values do not increase so greatly. However, this exception in the case of *Patiria* may be due in part to the fact that measurements were not made at the lower temperatures, and could not be, because as previously described, cold

TABLE II

Temperature Coefficients (Q_{10}) for Different Ranges of Temperature

Temperature interval	Q_{10} values			
	Dendraster	Strongylocentrotus	Patiria	Urechis
°*C.*				
2°– 4°.........	6.7	12.4	—	—
2°– 5°.........	7.0	8.1	—	8.2
2°– 7°.........	5.4	5.0	—	6.0
5°–10°.........	3.1	2.3	2.1	4.3
7°–12°.........	2.1	2.2	2.5	3.9
10°–15°.........	1.5	2.7	1.8	3.9
12°–17°.........	2.1	2.5	1.6	3.1
15°–20°.........	2.0	1.4	1.5	1.5
17°–22°.........	1.3	1.3	1.3	1.6
20°–25°.........	− 2.3	− 1.3	1.2	2.2
22°–27°.........	− 3.7	− 2.7	1.4	2.1
25°–28°.........	− 2.4	—	1.7	—
25°–30°.........	—	− 2.4	—	2.3
27°–32°.........	—	− 2.3	—	2.8
30°–32°.........	—	− 4.7	—	—
30°–35°.........	—	—	—	1.7

alone induces a sort of cytoplasmic withdrawal from regions of the egg surface leaving the preformed membrane partially separated from the egg. This involves another phenomenon.

It seems safe then to conclude that the rate of the processes leading to the development of the fertilization membrane in eggs of the four species is not limited by metabolic rate throughout most of the temperature range of fertilization. In the middle ranges it may be limited by metabolic rate.

The Q_{10} values do not indicate what the limiting reaction is, but rather, what type of reaction it could or could not be. As for the types of non-metabolic factors which limit the rate at low temperatures where the coefficients are high, e.g., $Q_{10} = 6.0$ to 12.0 and at temperatures where the coefficients are low, e.g., $Q_{10} = 1.3$ to 1.5, reasonable probabilities are viscosity in the former case and diffusion, imbibition, or some other physical factor in the latter. Viscosity, or the beginning of coagulation, may also be the limiting factor where the coefficients are negative at higher temperatures. The coefficients and their trends suggest that the underlying reactions are of the same general sort as those which limit the rate of cell division [see particularly Loeb and Wasteneys (1911), Fauré-Fremiet (1913), and in corresponding temperature ranges, certain stages in mitosis, Ephrussi (1927)].

In *Dendraster* and *Strongylocentrotus*, in contrast to *Patiria* and *Urechis*, the Q_{10} values become negative at higher temperatures. This is merely another way of describing what may be seen more directly by comparing the time-temperature curves (Fig. 1), namely, that the reaction slows up as the temperature is increased above 22° C. in the one case, while proceeding with continually increasing rate to the maximum fertilization temperature in the other. This sharp functional difference confirms the evidences already given (Chase, 1935) that there are differences in parts at least of the reactions which lead to the development of the fertilization membrane in these different kinds of eggs. It seems reasonable, and is consistent with all of the results obtained, to conclude that in *Dendraster* and *Strongylocentrotus*, in contrast to *Patiria* and *Urechis*, a relatively large measure of active transformation of a precursor substance is included in the processes which precede the development of the fertilization membrane.[3] These steps are omitted in the case of the fertilization membrane in eggs of *Patiria* and *Urechis* because there is already a well-formed membrane on the unfertilized egg which is separated after the fertilization of the egg. It may be that rate retardation above

---

[3] This may involve precipitation, as suggested by Harvey (1914).

22° C. is absent in *Patiria* and *Urechis* because the affected egg activities are missing. Except for the reversal in the effect of temperature on the rate at 22° C. in two of the species, the temperature coefficients suggest comparable underlying processes in the four species.

SUMMARY

1. The activity of the spermatozoön is affected by extreme temperatures. At 2° C. the sperm of *Dendraster* and *Patiria* are feebly active while the sperm of *Strongylocentrotus* and *Urechis* are very active. The activity of the sperm increases with rise in temperature and is retarded at high temperatures. At 25° C. sperm of all species were very active, but at 28.5° C.–30° C. *Dendraster* and *Patiria* sperm became inactive. *Strongylocentrotus* and *Urechis* sperm became inactive at 35° C.–40° C.

2. At 2° C. unfertilized eggs of *Dendraster*, *Strongylocentrotus*, and *Urechis* were apparently unaffected while eggs of *Patiria* were activated by cold at temperatures of 8° C. and below. High temperatures caused partial activation of the eggs, usually followed by cytolysis. These effects occurred at and above the following temperatures: *Dendraster*, 29° C.; *Patiria*, 30° C.; *Strongylocentrotus*, 32° C.; and *Urechis*, 35° C.

3. The rate of the cortical reactions leading to the development of the fertilization membrane in eggs of the four species was retarded at low temperatures and, in the case of eggs of *Dendraster* and *Strongylocentrotus*, increased with rise in temperature up to 22° C. but decreased above 22° C. up to the maximum fertilization temperatures. In eggs of *Patiria* and *Urechis* the rate of the reaction increased steadily up to the maximum fertilization temperatures.

4. The effect of temperature on the rate of the cortical reactions in the different kinds of eggs affords a functional basis for grouping the eggs in two general categories with respect to the underlying reactions, which agrees entirely with other types of evidence in regard to the mode of the development of the fertilization membranes.

5. The temperature coefficients (Q_{10}) of the rates of the cortical reactions leading to the development of the fertilization membrane in eggs of *Dendraster*, *Strongylocentrotus*, *Urechis*, and to a lesser extent of *Patiria*, are not constant over the range of fertilization, but shift progressively over a wide range. At colder temperatures the coefficients attain values of 6.0 to 12.0 except in *Patiria*, whose eggs were not fertilized at low temperatures. At mid-temperature ranges the coefficients are 1.3 to 1.5 in the four types of eggs.

6. The coefficients indicate (see Table II) that the rate of the

cortical reaction is not limited by metabolic rate over most of the viable temperature range since over most of the range Q_{10} is not of the order of magnitude of 2.

7. It is suggested that in the cortical reactions, as in such developmental phenomena as the division of egg cells, in addition to metabolic factors, other factors such as viscosity, diffusion, imbibition, etc., are involved; and that over most of the viable temperature range, it is such factors which limit the rate of reaction.

BIBLIOGRAPHY

Bayliss, W. M., 1924. Principles of General Physiology, 4th edition. London, Longmans, Green, and Co.

Chase, H. Y., 1935. The origin and nature of the fertilization membrane in various marine ova. *Biol. Bull.*, **69**: 159.

Crozier, W. J., 1924. On biological oxidations as function of temperature. *Jour. Gen. Physiol.*, **7**: 189.

Ephrussi, Boris, 1927. Sur les coefficients de temperature des differentes phases de la mitose des oeufs d'Oursin (Paracentrotus lividus, Lk.) et de l'Ascaris megalocephala. *Protoplasma*, **1**: 105.

Fauré-Fremiet, E., 1913. Le cycle germinatif chez l'Ascaris megalocephala. *Arch. D'Anat. Micr.*, **15**: 435.

Fisher, W. K., and G. E. MacGinitie, 1928. A new echiuroid worm from California. *Ann. and Mag. Nat. Hist.*, Ser. 10, **1**: 199.

Fisher, W. K., and G. E. MacGinitie, 1928. The natural history of an echiuroid worm. *Ann. and Mag. Nat. Hist.*, Ser. 10, **1**: 204.

Greeley, A. W., 1902. Artificial parthenogenesis in starfish produced by a lowering of temperature. *Am. Jour. Physiol.*, **6**: 296.

Loeb, J., 1906. Versuche über den Chemischen Charakter des Befruchtungsvorgangs. *Biochem. Zeitschr.*, **1**: 183.

Loeb, J., 1910. How can the process underlying membrane formation cause the development of the egg? *Proc. Soc. Exper. Biol. and Med.*, **7**: 120.

Loeb, J., 1916. The Organism as a Whole. New York. G. P. Putnam's Sons.

Loeb, J., and H. Wasteneys, 1911. Sind die Oxidationsvorgänge die unabhängige Variable in den Lebenserscheinungen? *Biochem. Zeitschr.*, **36**: 345.

Loeb, J., and H. Wasteneys, 1915. Further experiments on the relative effect of weak and strong bases on the rate of oxidations in the egg of the sea urchin. *Jour. Biol. Chem.*, **21**: 153.

Snyder, C. D., 1908. A comparative study of the temperature coefficients of the velocities of various physiological reactions. *Am. Jour. Physiol.*, **22**: 309.

Snyder, C. D., 1911. On the meaning of variation in the magnitude of temperature coefficients of physiological processes. *Am. Jour. Physiol.*, **28**: 167.

SEASONAL MIGRATIONS OF THE WOOD–BORER LIMNORIA LIGNORUM (RATHKE) AT FRIDAY HARBOR, WASHINGTON

MARTIN W. JOHNSON

(*From the Scripps Institution of Oceanography of the University of California, La Jolla, California*)

INTRODUCTION

The present report follows a year of observations instituted to verify the apparent migrations of *Limnoria lignorum* during an investigation on seasonal settlement of sessile organisms at Friday Harbor, Washington in 1928–1929 (Johnson and Miller, 1935). Since regular seasonal migrations of *Limnoria* have not before been reported, it seemed advisable to carry out at least an additional year's work as a check. Consequently, test blocks were again put in place in 1930. Some of these were lost and the work was temporarily abandoned, and further delay followed my absence from the West Coast. The ensuing lapse of time may, however, prove of value in giving data on isolated years and thus introducing more extreme conditions, at least with respect to winter temperatures.

Limnoria lignorum is a widely distributed marine wood-boring crustacean which is frequently found in vast numbers in infected areas where environmental conditions, especially salinity, are favorable to its requirements. The animals attain a maximum length of only about 5 mm., and nearly 400 individuals (juveniles predominating) may be present in one cubic inch of heavily infected wood. The deterioration of wooden marine structures due to the ravages of this gribble is an item of considerable economic importance along the Pacific and Atlantic coasts of America and in many other parts of the world where marine structures are maintained. Its attack occurs on the surface of the wood where small burrows are made, forming channels following mainly the softer, less lignified portions of the grain. Thus, as the wood becomes highly porous and friable, it is easily eroded away and deeper wood is freshly attacked producing a characteristic wasting of the timber which is most pronounced near the low tide level.

It is generally believed that the dispersal of *Limnoria* is brought about mainly by incidental transportation on infected driftwood etc., and by occasional wanderings of free-swimming individuals. The data here given lead me to believe that the principal means of dispersal within an infected area is by a regular seasonal migration which is perhaps associated with the main breeding season. These migrations must not be understood as occurring over any considerable distances but rather as a matter of only a few meters or less. Mechanical transportation by drift of infected wood etc. still remains the only explanation for the spread of infection to more remote areas, for *Limnoria* is not a strong swimmer and does not possess the pelagic egg or larval stage which plays such an important rôle in the wide and rapid dispersal of many marine invertebrates. The eggs and young are few in numbers, about 10 to 23, and are retained in a ventral brood pouch of the female. When about 1.0 to 1.2 mm. long the young escape and immediately begin burrowing into the wood where they were born. Thus, though the reproductive power is relatively small, the hazards of a helpless pelagic existence are eliminated and the total population increases at a rapid rate. Destruction by natural enemies is apparently negligible, particularly while the animals remain in the burrows. The migrations referred to above, do, however, introduce a period of special hazard, but the short distances involved in swimming freely in the water must reduce this peril materially.

The author wishes to thank Dr. T. G. Thompson, Director of the University of Washington Oceanographic Laboratories, for laboratory facilities and equipment at Friday Harbor; and Dr. Lyman D. Phifer, Assistant Director, for 1934 temperature data.

METHODS

Seasoned Douglas fir boards, 9 cm. × 2 cm. × 35 cm., served as test blocks. Prior to being used, these were soaked for two weeks in sea water which had been heated to kill all larvæ. A set of two blocks constituted a series. The two blocks were attached one meter apart to a long wire which was suspended vertically from a beam under the Friday Harbor wharf where there was known to be a heavy infection of *Limnoria.* A weight attached to the wire below the lower block held it in position so that the upper block was a little above zero tide level and the lower block somewhat below this level (In the 1928–29 investigation it was found that nearly 90 per cent of the *Limnoria* settle at these levels). Horizontally the blocks were about a meter from the nearest piles.

The number of animals occurring on an infected block was determined by counting the burrows, and when infection was light all the burrows were counted, but during heavy attack only a limited portion was counted and the numbers computed from the size of the areas involved.

In order to obtain a census of size distribution which would be typical of the whole population, and not a selected one as appears to exist on newly populated test blocks, each month a small sample of wood was taken from an old infected wharf pile. These samples included a clean cut from the surface to unattacked wood. The wood thus removed was picked apart and all sizes of *Limnoria* recorded from the sample selected. In this operation it was necessary to use a dissecting microscope, for the young stages are quite colorless and can be seen against the wood only with difficulty.

The sexes are separable only on minute differences, hence in the examination for gravid females as an index to reproductive activity, no separation of sexes was made and all specimens of adult size (3.4 mm. and over) were considered.

It should be noted that the test pieces were always in a shaded position and, aside from a few filamentous diatoms, no vegetation which would interfere with settlement occurred on the wood. A number of sessile animals, mainly hydroids, bryozoa, barnacles, and tube worms were found particularly in the spring and autumn, but not in numbers sufficient to interfere with the activities of *Limnoria*.

Table I

Seasonal settlement of Limnoria lignorum in two-month periods from September 5, 1933 to September 8, 1934

Period of exposure	Sept. 5 to Nov. 7	Nov. 7 to Jan. 5	Jan. 5 to Mar. 7	Mar. 7 to May 9	May 9 to July 7	July 7 to Sept. 8
Upper block	8	32	348	605	146	16
Lower block	16	41	314	437	149	26
Totals	24	73	662	1,042	295	42
Percentage of yearly settlement	1.12	3.42	31.00	48.80	13.80	1.97

Results

Examination of the test blocks which were exposed to settlement and attack over periods of two and three months showed a marked increase in the number of *Limnoria* settling on the blocks during late winter and spring months. The remaining portion of the year was relatively free from settlement but there was no period of complete immunity.

Two-month Series.—The seasonal distribution as indicated by the number of animals occurring on the test blocks at the end of each two months of exposure is given in Table I. A study of these data shows that the first significant increase in settlement occurred sometime between January 5 and March 7, when the blocks for this period were removed. The next two months, March 7 to May 9, witnessed a yet greater settlement, as is evidenced by examination of the blocks removed May 9 when the greatest number of individuals was counted. During the next period, ending July 7, there was a definite decrease in numbers. The decline continued to the end of the investigation on September 8. The period of minimum migration covers September and October, when only 1.12 per cent of the total yearly settlement took place, but July, August, November and December also witness reduced migratory activity. The maximum migration may be assumed to have occurred between the dates having the highest counts, that is, some time in March or April, the two-month period having 48.80 per cent of the yearly settlement. It is instructive to note that 93.60 per cent of the total settlement took place over the six-month period, January 5 to July 7, and that 79.80 per cent was for the four months from January 5 to May 9. The numbers recorded when the blocks were removed and examined represent a two-month accumulation. Hence, when these blocks cover a period at the beginning of increasing migration, it is reasonable to believe that the greatest number of *Limnoria* settled on the blocks toward the end of that period. Conversely, on a waning migration, after the maximum is just past, the first portion of the period following would receive the greater number, provided decline be uniform. Thus the total period over which migration was most pronounced is somewhat narrowed, and January and also June become much less important than February and May, the two months which were included with them respectively in the periods of exposure to settlement immediately before and after the crest of the migration.

TABLE II

Seasonal settlement of Limnoria lignorum in three-month periods from September 5, 1933 to September 8, 1934

Period of exposure	Sept. 5 to Dec. 6	Dec. 6 to Mar. 7	Mar. 7 to June 9	June 9 to Sept. 8
Upper block	18	572	1,316	118
Lower block	25	335	1,400	207
Totals	43	907	2,716	325
Percentage of yearly settlement	1.08	22.65	68.00	8.15

Three-month Series (Table II, Fig. 1).—These show essentially the same as the two-month series. The longer period of exposure makes an interpretation of the data more difficult and uncertain, but they indicate a continuance of rather active migration through May. By reference to the two-month series, it will be noted, however, that the month of May did not experience a very heavy settlement and it is probable that that month contributed only moderately to the maxi-

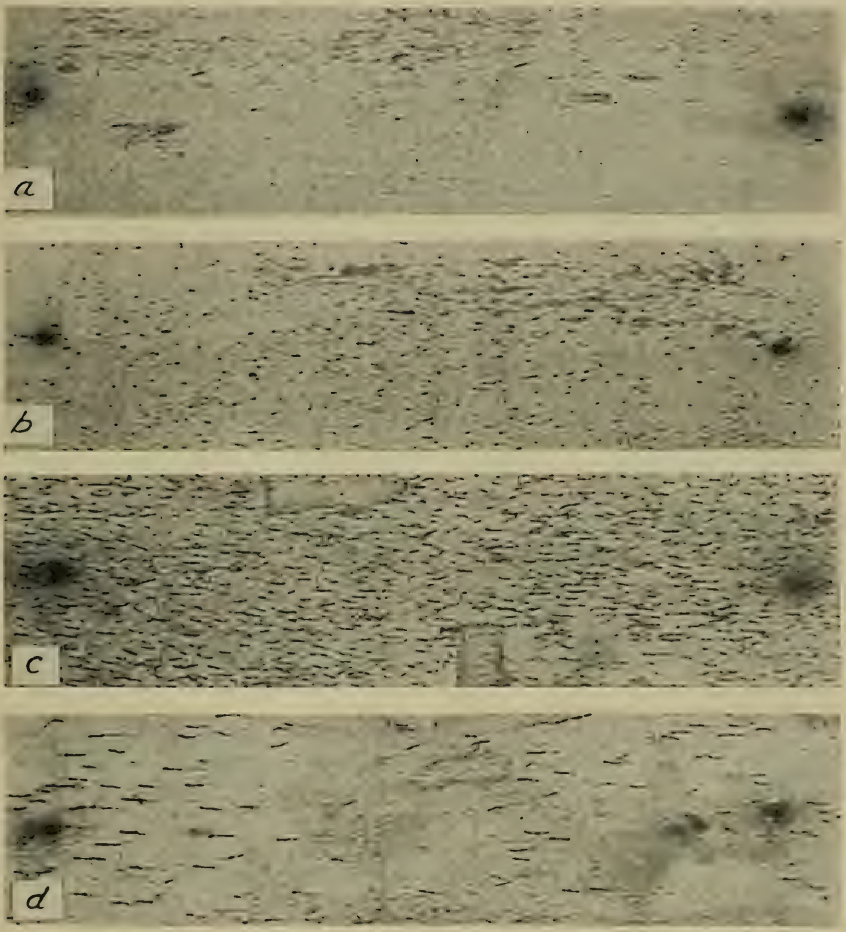


FIG. 1. The lower blocks of the four series which were exposed to settlement of *Limnoria lignorum* over the three-month periods indicated:

a. September 5, 1933, to December 6, 1933;
b. December 6, 1933, to March 7, 1934;
c. March 7, 1934, to June 9, 1934;
d. June 9, 1934, to September 8, 1934.

mum which occurred from March 7 to June 9. December is similarly but more completely eliminated as an important month in the period December 6 to March 7, with the result that most of the 90.65 per cent of settlement which occurred on the exposures covering the six months from December 6 to June 9 must have taken place during January, February, March, April, and May, with the importance of January doubtful, and May more or less restricted.

A comparison with the three-month series of 1928–29 (Johnson and Miller, 1935) shows in general a very close agreement. During that year, however, pronounced migration appears to have begun a little later in the winter and continued later in the spring, making May also an important migratory month and to a much lesser degree also June. In selecting (from the over-lapping series of 1928–29) a succession of series covering three-month periods from October 1, 1928 to September 26, 1929, we find the following percentage of total yearly settlement for the periods indicated: October 1 to January 1, 1.98 per cent; January 1 to March 28, 27.93 per cent; April 1 to July 2, 63.92 per cent; July 2 to September 26, 6.17 per cent. From this it is seen that 91.85 per cent of the total yearly settlement occurred over the six-month period from January 1 to July 2. It is obvious, however, that January was not an important migratory month that year and contributed but little to the total settlement of the six-month period.

In 1928–29 active migration occurred on a rising temperature which ranged from about 6.55° C. to 9.99° C. In 1933–34 the average monthly temperature range over the most active period was from 7.71° C. to 9.42° C. The minimum temperature recorded for 1929 was on February 1, at which time it was 6.4° C., whereas in 1934 a minimum of only 7.52° C. was recorded on February 22. The lower temperatures of 1928–29 may be responsible for the tendency to later migration during that year (See Table V). Salinities at Friday Harbor are always within the limits required by *Limnoria* (Kofoid and Miller et al., p. 327, 1927).

Size Groups.—Measurements of specimens occurring on the test blocks revealed only mature or submature animals except in a few instances where also very young specimens were recorded from the three-month blocks removed June 9 and September 8. These young were, however, few in number and doubtless were from broods recently released.

The ratio of young to the intermediate and adult groups gave a very different picture in the samples which were collected from the population of old infected wharf-piles. Three size groups were considered, namely, 2.0 mm. or less; 2.1 mm. to 3.4 mm.; 3.5 mm. or over.

The first group comprises recently or newly hatched young, the second group, older juveniles and possibly some small mature males, and the third group, only adults. From Table III it will be seen that the youngest group was the dominant one throughout the year (no sample available for August). During late summer and early winter there is a gradual relative decrease as the specimens of this group become older and enter into the group of intermediate size. During March, (April?) and May, however, the percentage of very young is noticeably increased. The adult group remains relatively constant, apparently as a result of animals passing over from the intermediate group at all seasons at a rate which masks any decline which might otherwise be apparent due to death rate. Since the trend of the percentages of the youngest group is a function of the other two groups, it is influenced by such changes as death rate of the adults, or migration of the older members of the intermediate group, but the relationship which it bears to the occurrence of gravid females indicates that it must also represent actual population changes in the group.

TABLE III

Limnoria lignorum.—Size groups from old wharf-pile population

Date	Total no. examined	2 mm. or less	Percentage of total	2.1 mm. to 3.4 mm.	Percentage of total	3.5 mm. or over	Percentage of total
1933							
Oct. 5	103	65	63.11	20	19.41	18	17.48
Nov. 7	127	62	48.88	32	25.20	33	25.94
Dec. 6	206	104	50.48	64	31.07	38	18.44
1934							
Jan. 5	101	50	49.50	35	34.65	16	15.84
Feb. 8	251	114	45.42	97	38.64	40	15.93
Mar. 7	291	172	59.10	86	29.55	33	11.34
Apr. 7	441	187	42.40	126	28.60	128	29.00
May 9	348	251	72.12	37	10.63	60	17.24
June 9	125	72	57.60	36	28.80	17	13.60
July 7	97	56	57.73	14	14.43	27	27.83
Aug.	—	—	—	—	—	—	—
Sept. 8	229	123	53.71	66	28.82	40	17.46

Gravid Females.—A record of the seasonal distribution of specimens carrying eggs or young in the brood pouch is given in Table IV. Gravid specimens were found only in the group measuring over 3.4 mm. long. It is evident that gravid females do occur throughout the year, that few are found in January and February, and that especially large numbers are gravid in early April and May, and the data suggests also a small increase in November and December. In 1928–29 it was also found that gravid specimens were present in each month of the year, with a minimum in February and early March and with significant numbers through spring, summer, and autumn. In that year there was indicated a rise following the main migratory season. In 1933–34 the reproductive activity appears to be more closely correlated with the maximum migratory activity but still somewhat lagging. The low temperatures of February and March of 1929 may account for the scarcity of gravid females in February and early March of that year, whereas in 1934 the fewest gravid specimens were found in January and February, the two months of lowest average temperature during that year.

Table IV

Limnoria lignorum—Gravid females

Date	Population from old wharf-piles		Population from two-month test blocks		Population from three-month test blocks		Total examined all populations	Percentage of gravid females in adult group
	No. adults examined	No. gravid females	No. adults examined	No. gravid females	No. adults examined	No. gravid females		
1933								
Oct. 5	55	6	—	—	—	—	55	10.91
Nov. 7	33	11	16	3	—	—	49	28.57
Dec. 6	38	11	—	—	16	4	54	27.78
1934								
Jan. 5	16	1	26	0	—	—	42	2.39
Feb. 8	40	3	—	—	—	—	40	7.50
Mar. 7	33	4	10	0	10	3	53	13.20
Apr. 7	101	49	—	—	—	—	101	48.50
May 9	60	18	45	5	—	—	105	21.90
June 9	17	3	—	—	65	18	82	25.60
July 7	27	4	38	9	—	—	65	20.00
Aug.	—	—	—	—	—	—	—	—
Sept. 8	40	13	23	5	60	11	123	23.58

Kofoid (1921, p. 52) found eggs and young throughout the year in San Francisco Bay, but no definite breeding season was established. It was suggested later (Kofoid and Miller et al., 1927, p. 329) that this lack of a definite reproductive season may be due to the small seasonal variation in temperature, i.e., approximately 8° C. Coker (1923), working at Beaufort, North Carolina, reports a definite breeding season which is correlated with a temperature of 14° C. At Beaufort the temperature range is given as about 20° C. At Friday Harbor the temperature range is only about 7° C. Henderson (1924, p. 321) observed gravid females at St. Andrews, New Brunswick, during July, August, September, October, and to a much lesser extent also in November and December. The other months were not investigated.

Discussion

From the above data it is quite evident that we are dealing with a period of active migration of *Limnoria*. The test blocks could be reached only by swimming animals or by chance transportation on driftwood or tidal currents. The orderly distribution through the seasons on several isolated blocks, and the uniformity of age in the population excludes the possibility of chance.

This migration appears to be a preliminary or complementary activity associated with the main reproductive season. It is reasonable to believe that, unless the death rate nearly equals that of reproduction, some movement of population must take place when the numbers become intolerable for an area which is constantly being diminished by the ravages of the population. During this migration many individuals no doubt move to already infected wood. Nevertheless there would be a general thinning out in the densely populated communities, and a large number of the migrants and their progeny would become established in more favorable habitats on sparsely populated or new timbers. The migrants represent that element of the population which is approaching or has just reached maturity, and but few, if any, of these appear to be gravid at the time of settling on the new habitat. This is brought out by the relatively small number of gravid specimens found on the two-month test blocks and also by the relative scarcity of very young

Table V

Temperature ranges at Friday Harbor during most active migratory period of Limnoria lignorum in 1929 and 1934

1929	Maximum ° C.	Minimum ° C.	Monthly average	1934	Maximum ° C.	Minimum ° C.	Monthly average
Jan.	7.8	7.1	7.53	Jan.	7.76	7.61	7.70
Feb.	6.7	6.4*	6.55	Feb.	7.82	7.52†	7.71
March	7.4	6.8	7.12	March	8.50	7.70	8.02
April	8.0	7.5	7.80	April	9.22	8.20	8.71
May	9.7	8.5	8.97	May	10.28	8.82	9.42
June	10.6	9.4	9.99	June	11.78	9.98	10.69
July	11.5	10.2	10.75	July	11.42	10.10	10.66

* February 1. † February 22.
1929 data from Thompson and Johnson 1930.

animals even on the three-month blocks. Temperatures at Friday Harbor during the most active migratory period (See Table V) were perhaps sufficiently low to induce a slow incubation, but two months, or at any rate three months would doubtless suffice for incubation, and one would expect to find large numbers of young on the blocks had the females been gravid at the time of settling.

Kofoid and Miller et al. (1927, p. 320) found a very regular and uniform infection of untreated test timbers exposed to *Limnoria* settlement in San Francisco Bay. This was true during every month of the year, and long exposures showed regular increasing severity of attack. They call attention to the problematic nature of the methods of dispersal but do not consider free-swimming a factor of much importance. In extensive tests of the reactions of *Limnoria* to wood and wood extractives, they found no evidence of a chemotropism which would aid the swimming animals in locating and recognizing submerged wood. Perhaps such a chemotropic mechanism is actively functional only at the age when active migration occurs.

Investigations in other areas also have not given evidence that local dispersal of *Limnoria* is due mainly to seasonal migrations. This may indicate an absence of pronounced seasonal variation in migratory activity in the localities investigated. There is, however, also some likelihood of the activity being overlooked, for studies pertaining to *Limnoria* have often been incidental to general wood-boring investigations where methods were designed to give information mainly on the molluscan borers or simply to determine the durability of different woods or preservatives. The effect of seasonal migrations may go unnoticed as a result of allowing test boards to remain in the water so long that population increases have occurred due to reproduction by the animals established on the boards. Thus the numbers of those actually settling on the boards as migrants would become masked by the large numbers resulting from reproduction, and also migrants may become discouraged from settling on already heavily populated wood. It is conceivable that the effect of swimming migration may also be largely obliterated by animals crawling from infected wood to conveniently placed test blocks. Commonly, test pieces are placed directly in contact with already infected wood, which may be a wharf-pile or a frame of wood specially constructed to support successive series of test boards.

Much effort has been directed towards devising means of protecting wooden piles and other marine structures against the devastating attacks of this and other marine borers. Creosote and various paints or surface applications offer a greater or lesser degree of protection, but at best this is not absolute or permanent, and because of the expense involved in utilizing creosoted timbers, many marine structures are built of untreated materials, and may receive little or no subsequent protective treatment. Such structures are necessarily short-lived in areas where borers are abundant.

From the foregoing data on the migratory habits of *Limnoria*, one is led to believe that when the nature of wooden marine structures does not preclude treatment, the most efficient time to make a protective application would be at the beginning of the main migratory season. Thus the minimum amount of leaching of the protective coating would have occurred before the period when it is most needed to repell the invasion of the animals. Other things being equal, unprotected timbers submerged in June or July should show much less deterioration at the end of a year than similar timbers submerged in February or March. The latter would suffer not only an early intensive attack of the migrants, but later also that of their young. The former, on the other hand, would have a six-month period of lighter early attack and subsequently fewer young produced to consume the wood over the last half of the year.

SUMMARY

1. Wooden test pieces were exposed to settlement of *Limnoria lignorum* in successive two and three-month periods from September 5, 1933 to September 8, 1934.

2. Monthly collections of wharf-pile populations were examined to determine the seasonal fluctuations in age groups, and in the occurrence of gravid females.

3. A marked seasonal variation in the numbers of *Limnoria* settling on the test blocks was found, and since the blocks could be reached only by swimming animals, it is believed to represent a definite seasonal migration which is associated with the main breeding season.

4. On the two-month test pieces, 93.60 per cent of the total yearly settlement of *Limnoria* occurred over a six-month period from January 5 to July 7; and 48.80 per cent settled from March 7 to May. 9. The three-month pieces showed essentially the same distribution.

5. The most active migration took place during a temperature range of about 7.71° C. to 9.42° C.

6. There was a predominance of young *Limnoria* throughout the year, but the greatest percentage of these was evident during the spring months.

7. Gravid females doubtless occurred throughout the year (no data for August), with a maximum in April and May and a minimum in January and February, the two months with the lowest temperatures.

8. As an aid to better protection against *Limnoria* attack on submerged timbers, it is suggested that the most effective time to apply protective paints etc. is at the beginning of the main migratory season.

BIBLIOGRAPHY

COKER, R. E., 1923. Breeding habits of Limnoria at Beaufort, North Carolina. *Jour. Elisha Mitchell Sci. Soc.*, **39**: 95.

HENDERSON, J. T., 1924. The gribble: A study of the distribution factors and life-history of Limnoria lignorum at St. Andrews, N. B. *Contr. Can. Biol.*, *N. S.*, **2**: 309.

JOHNSON, MARTIN W., AND ROBERT C. MILLER, 1935. The seasonal settlement of shipworms, barnacles, and other wharf-pile organisms at Friday Harbor, Washington. *Univ. of Washington Publ. in Oceanography*, **2**, No. 1: 1.

KOFOID, C. A., 1921. The marine borers of the San Francisco Bay region. Report on the San Francisco Bay Marine Piling Survey, pp. 23–61.

KOFOID, C. A., AND R. C. MILLER et al., 1927. Biological Section, Final Report of the San Francisco Bay Marine Piling Committee, pp. 188–343.

THOMPSON, T. G., AND M. W. JOHNSON, 1930. The sea water at the Puget Sound Biological Station from September 1928 to September 1929. *Publ. Puget Sound Biol. Sta.*, **7**: 345.

FEEDING HABITS AND PHARYNGEAL STRUCTURE IN STENOSTOMUM

JOHN W. NUTTYCOMBE AND AUBREY J. WATERS

(*From the Department of Zoölogy, University of Georgia*)

Introduction, Materials and Methods

A comparative study of pharyngeal structure was suggested by the diverse feeding habits exhibited by certain species of *Stenostomum.* The species selected for study were *S. grande* Child (1902), *S. virginianum* Nuttycombe (1931) and *S. predatorium* Kepner and Carter (1931).

S. grande typically feeds upon relatively large masses of debris which may contain bacterial glea, protozoa, rotifers, unicellular plants, and the like. It will also occasionally ingest whole, smaller specimens of its own or other species of *Stenostomum.* The mouth is capable of enormous distension. The food taken into the pharynx is retained momentarily and then passed through the pharyngo-enteric sphincter into the enteron.

The food of *S. virginianum* consists almost entirely of small, motile animals such as protozoa, rotifers, and whole, smaller members of its own or other species of *Stenostomum.* In the capture of its prey the mouth is quickly opened and the pharynx is dilated almost simultaneously. The suction created by the dilation draws the prey into the pharynx where it is imprisoned by the contraction of the oral sphincter. Usually the captured organism is retained in the pharynx for several seconds before it is forced back into the enteron by the simultaneous opening of the pharyngo-enteric sphincter and contraction of the intrinsic, circular musculature of the pharyngeal wall.

S. predatorium appears to exist entirely upon the tissues of other species of *Stenostomum.* We have for several years maintained cultures of this species by feeding it upon *S. tenuicauda.* *S. predatorium* feeds in a peculiar manner. It applies its "lips" to the surface of *S. tenuicauda* and, through the combined "sucking" action of its pharynx and constriction of its oral sphincter, is capable of drawing in and pinching off pieces of its prey which may then be passed into the enteron. It seldom, if ever, ingests a whole animal as do both *S. grande* and *S. virginianum.* Instead it takes a small piece of its prey whenever it comes in contact with it.

Thus we have here three species which differ sharply in feeding habits and which we have found to exhibit a high degree of morphological, pharyngeal adaptation.

Representatives of the three species were studied in the living condition and in fixed preparations. For fixation Gilson's and modified (double quantity of osmic acid with addition of 10 per cent urea by weight) Meeve's fluids were used. Fixed material was embedded in rubber-paraffin and sectioned at 5 micra. Heidenhain's haematoxylin was used as a stain.

GENERAL MORPHOLOGY OF THE PHARYNX

The pharynx in the genus *Stenostomum* is essentially a simple, tubular structure equipped at both oral and enteric ends with sphincter muscle fibers. The intrinsic musculature of the pharyngeal wall is composed of circular and longitudinal layers. Extrinsic, radial muscle fibers anchor the pharynx to the body wall. In most species unicellular glands are associated with the pharyngeal region.

COMPARATIVE MORPHOLOGY

Comparison of Living Specimens.—Studies of the pharynx in living specimens of these three species of *Stenostomum* show sharp differences in gross morphology.

In *S. grande* (Plate I, Fig. 1) the pharynx is marked off rather definitely by a transverse constriction into an anterior region, which is covered externally by unicellular glands, and a posterior (œsophageal) region lacking glands. The radial musculature in this species is relatively undeveloped and consists of scattered fibers running from the pharyngeal wall to the body wall.

Explanation of Plate I.

FIG. 1. Dorsal aspect of pharyngeal region of living *S. grande*.
FIG. 2. Dorsal aspect of pharyngeal region of living *S. virginianum*.
FIG. 3. Dorsal aspect of pharyngeal region of living *S. predatorium*.
FIG. 4. Transverse section through the anterior pharyngeal region of *S. grande*.
FIG. 5. Transverse section through the mid-region of the pharynx of *S. virginianum*.
FIG. 6. Transverse section through the posterior pharyngeal region of *S. predatorium*.

List of abbreviations used in Plates I and II: *a*, special oral sphincter; *b*, cerebral commissure; *c*, circular muscle layer of the pharynx; *d*, concentrated mass of radial fibers; *e*, epidermis; *en*, enteron; *ep*, œsophageal portion of the pharynx; *f*, free-cells of the pseudocœl; *g*, pharyngeal gland cells; *h*, intrinsic musculature (longitudinal and circular); *l*, longitudinal muscle layer of the pharynx; *m*, mouth; *n*, protonephridial tube; *o*, oral sphincter; *oe*, oral epithelium; *p*, pharynx; *pe*, pharyngeal epithelium; *ps*, pharyngo-enteric sphincter; *r*, radial muscle fibers; *s*, special, heavy, radial fibers; *t*, testis. Epidermal cilia and rhabdites omitted in all figures.

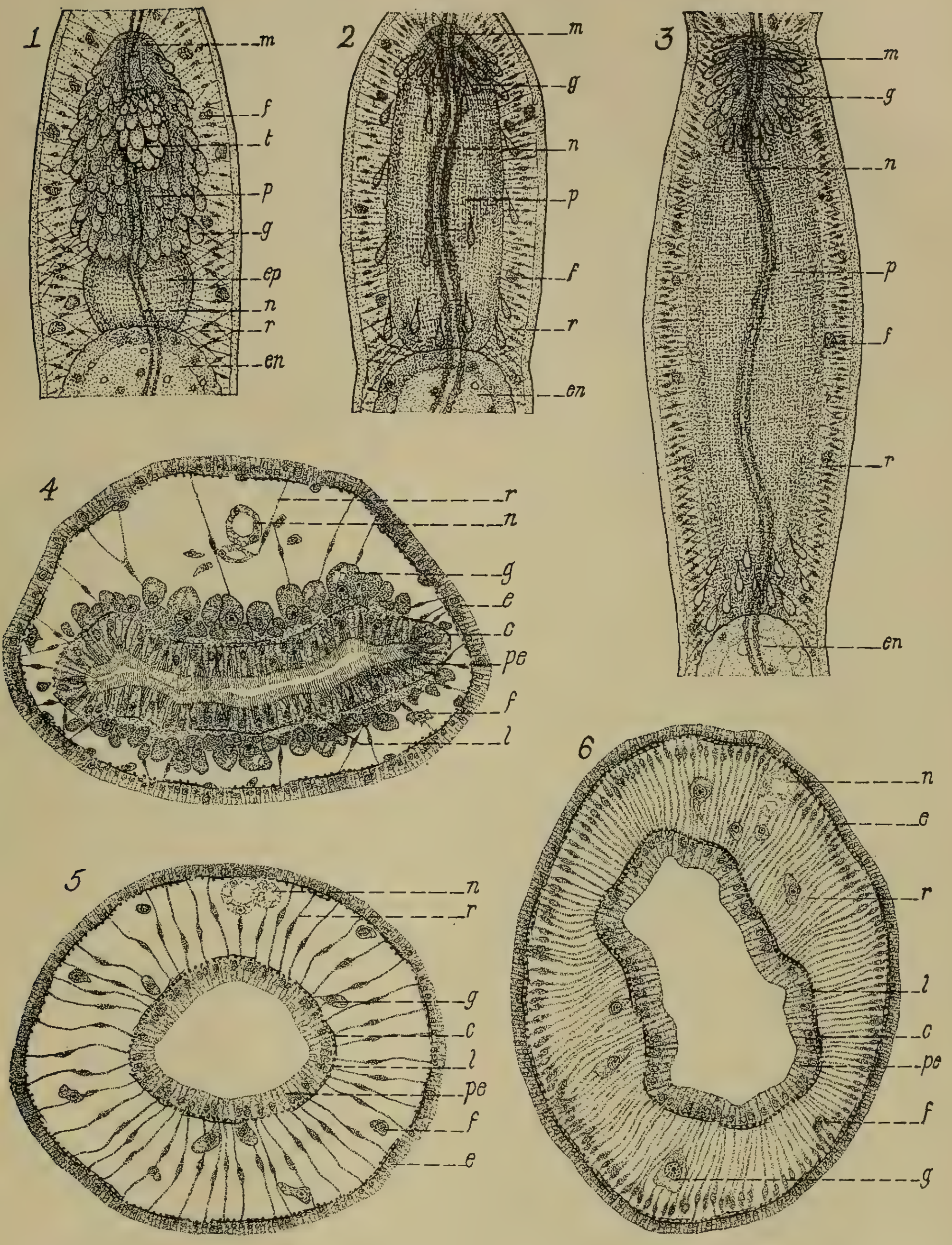

1
m
f
t
p
g
ep
n
r
en
2
m
g
n
p
f
r
en
3
m
g
n
p
f
r
en
4
r
n
g
e
c
pe
f
l
5
n
r
g
c
l
pe
f
e
6
n
e
r
l
c
pe
f
g

Plate I

In *S. virginianum* (Plate I, Fig. 2) the pharynx shows no regional differentiation of its wall and is of rather uniform diameter throughout its length. The unicellular glands are smaller and are distributed sparsely over the entire surface of the pharyngeal wall. The radial musculature is much more elaborate than that of *S. grande.*

S. predatorium (Plate I, Fig. 3) has a relatively much longer pharynx than either of the preceding species. Its wall shows no regional differentiation. Large unicellular glands are in this species confined to the oral and enteric ends of the pharynx. The radial musculature is the most elaborate found in the American species of the genus—the fibers are so heavy and so closely packed as to almost obscure the pharynx which they support. In the figure only the lateral, radial fibers are shown.

Comparative Histological Structure.—Transverse and sagittal sections of the pharyngeal region of the three species clearly illustrate the main histological differences.

Transverse and sagittal sections of this region in *S. grande* are shown respectively in Plate I, Fig. 4, and Plate II, Fig. 1. The transverse section was taken through the anterior pharyngeal region. The ciliary coat of the pharyngeal epithelium (*pe*) is extremely dense in this region and the epithelium itself shows groups of chromatic lines. Just outside the basement membrane of the epithelium may be seen the cut ends of the fibers (*l*) composing the longitudinal layer of the intrinsic, pharyngeal musculature and just external to this layer lies the circular layer (*c*) of the intrinsic pharyngeal musculature. The bundles of the longitudinal layer are considerably heavier than those of the circular layer. Lying in the pseudocœl may be seen the numerous, large, unicellular, pharyngeal glands (*g*) characteristic of this region. The radial, pharyngeal musculature is composed of a few relatively weak fibers (*r*) passing from the wall of the pharynx to the body wall. These fibers frequently show branching. The sagittal section of *S. grande* brings out some additional features. The epithelium of the posterior (œsophageal) pharyngeal region (*ep*) is thinner and its ciliary coat much less dense. There is not any great concentration of circular muscle fibers in either the oral or the pharyngoenteric sphincter regions. The points of attachment of the radial muscle fibers are more numerous just back of the constriction representing the line of demarcation between the anterior and œsophageal, pharyngeal regions. Associated with the dorsal lip may be seen the bases of a concentrated mass (*d*) of radial fibers. The distal ends of some of these fibers are attached dorsally and laterally to the body wall at the level of the mouth and still others are attached to the body

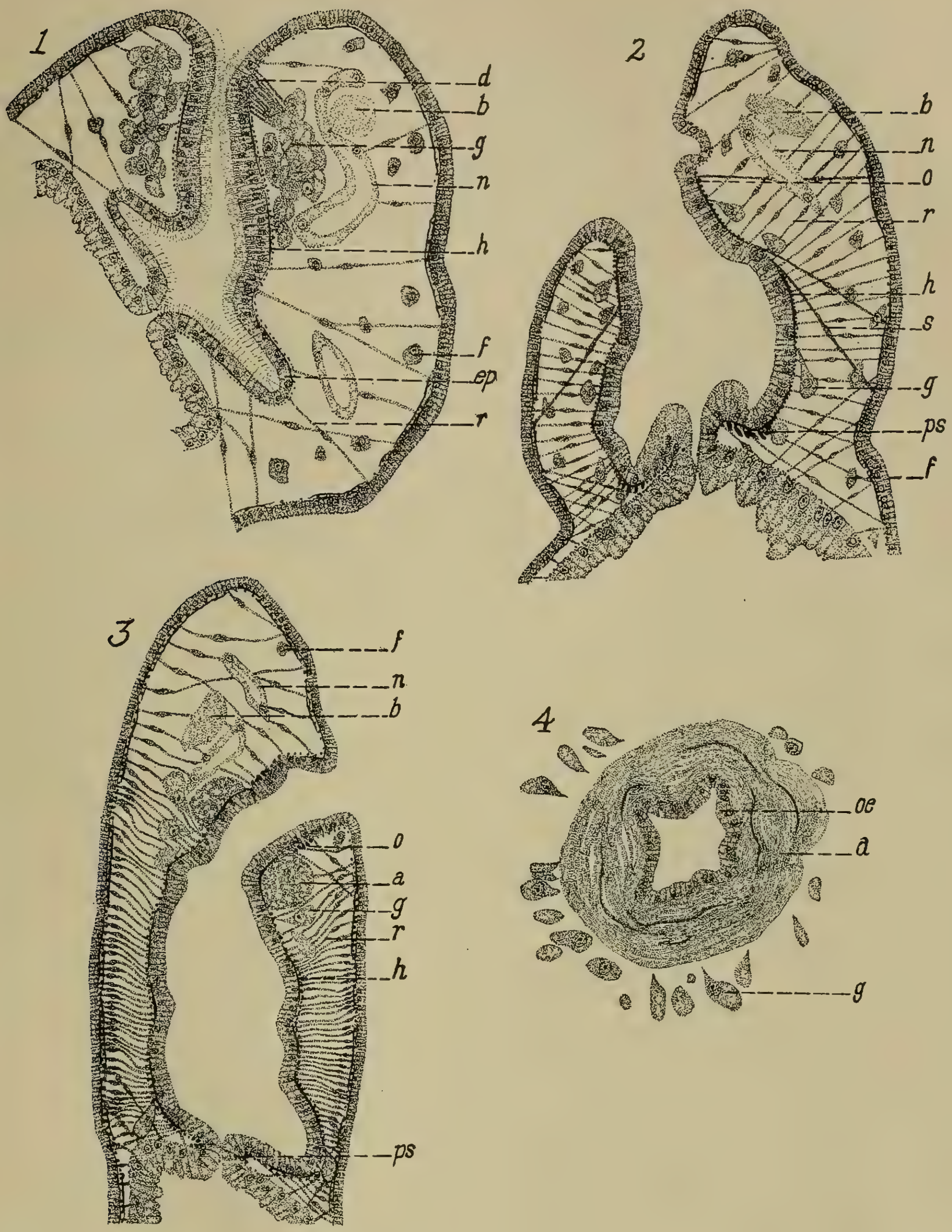

1
d
b
g
n
h
f
ep
r
2
b
n
o
r
h
s
g
ps
f
3
f
n
b
o
a
g
r
h
ps
4
oe
a
g

PLATE II

FIG. 1. Sagittal section through the pharynx of *S. grande*. Testis not shown.
FIG. 2. Sagittal section through the pharynx of *S. virginianum*.
FIG. 3. Sagittal section through the pharynx of *S. predatorium*.
FIG. 4. Slanting, frontal section through the special, oral sphincter of *S. predatorium*.

wall at more posterior levels. The distal attachments do not show in the section illustrated. These fibers are responsible for the extreme extensibility of the oral opening.

The transverse section (Plate I, Fig. 5) through the middle of the pharynx of *S. virginianum* gives us a very different picture. The ciliary coat of the pharyngeal epithelium (*pe*) is so fine as to appear lacking in many sections. The most significant differences, however, are seen in the radial musculature and pharyngeal glands. The radial muscle fibers (*r*) in *S. virginianum* are numerous and well-developed. The pharyngeal glands (*g*) are few and small. The sagittal section (Plate II, Fig. 2) of this species shows to even better advantage the distinctive features of its pharyngeal structure. The pharyngeal epithelium is of uniform thickness throughout the length of the pharynx. The intrinsic, longitudinal musculature shows about the same degree of development as in *S. grande* but the intrinsic, circular layer shows unusually large bundles in both the oral (*o*) and pharyngo-enteric sphincter (*ps*) zones. The radial muscle fibers (*r*) are numerous and powerful. In addition to the regular radial fibers there are several unusually heavy bundles (*s*) so placed as to be capable of making possible a greater extension of the oral opening. The small pharyngeal glands (*g*) are not localized in the anterior half of the pharynx as are the corresponding but larger glands in *S. grande.*

The transverse section (Plate I, Fig. 6) near the posterior end of the pharynx of *S. predatorium* shows an epithelium (*pe*) which is apparently unciliated. The intrinsic, pharyngeal musculature at this level is developed to approximately the same extent as is the corresponding musculature in *S. grande* and *S. virginianum.* The radial musculature, however, is enormously more highly developed than that of either of the other species. The nuclei of the radial fibers (*r*) are not located indiscriminately but are placed near the point at which the fiber is inserted on the body wall. The large, unicellular, pharyngeal glands (*g*) are prominent features at this level of the pharynx. The sagittal section (Plate II, Fig. 3) of *S. predatorium* shows a uniform, pharyngeal epithelium. The intrinsic, pharyngeal musculature shows some strengthening in its circular bundles at both the oral (*o*) and pharyngo-enteric sphincter (*ps*) regions. In addition to the strengthened bundles of the regular circular layer at the oral level there is an extremely powerful, special, oral sphincter (*a*). This ring of muscle surrounds the anterior opening into the pharynx and can be seen in sagittal section as a mass above and below the oral end of the pharynx. A slanting, frontal section of this sphincter is shown in Plate II, Fig. 4. This special sphincter is the most powerful muscular unit occurring in any of the American species of the genus. The sagittal section also shows the extremely powerful, radial musculature (*r*). The large, unicellular glands (*g*) are confined to the anterior and posterior pharyngeal regions.

SUMMARY

The variations in feeding habits exhibited by different species of the genus *Stenostomum* are largely dependent upon adaptive modification of pharyngeal structure.

The pharynx of *S. grande* is well adapted to the ingestion of large masses of relatively inert food material. The heavy band of radial muscles attached at the lip region is so arranged that it is capable of causing the enormous distension of the oro-pharyngeal region thus making possible the ingestion of very large masses. The heavy ciliary coat of the anterior, pharyngeal region is adapted to aid in the backward propulsion of such large masses into the thin-walled, distensible, œsophageal region of the pharynx from whence the mass may be forced through the pharyngo-enteric sphincter by the activity of the intrinsic, pharyngeal musculature.

In *S. virginianum*, the food of which is largely made up of small, highly motile prey, we find well-developed oral and pharyngo-enteric sphincters which allow the imprisonment of the captured organisms in the pharynx. There are several large bundles of radial fibers so disposed as to make possible a quick distension of the lips, and the sudden contraction of the powerful, radial, pharyngeal musculature enables this species to generate sufficient suction to draw its prey into the pharynx.

The pharynx of *S. predatorium* shows two prime structural adaptations. The radial musculature is strong enough to produce sufficient suction to draw into the mouth the tissues of another species of *Stenostomum* and the special oral sphincter by its contraction is capable of pinching off from the body of the prey the mass of tissue drawn into the pharynx.

In general it may be said for all species that the extrinsic, radial musculature primarily determines the method by which the food is drawn into the pharynx and the general intrinsic musculature of the pharyngeal wall is responsible for the transmission of the food to the enteron.

We have been unable to assign any differential, functional capacity to the pharyngeal glands in spite of the fact that sizes and distribution in the three species are sharply different.

LITERATURE

CHILD, C. M., 1902. Studies on regulation. I. Fission and regulation in Stenostoma. *Arch. f. Entw.-mech.*, **15**: 187.

KEPNER, W. A., AND J. S. CARTER, 1931. Ten well-defined new species of Stenostomum. *Zool. Anz.*, **93**: 108.

NUTTYCOMBE, J. W., 1931. Two new species of Stenostomum from the southeastern United States. *Zool. Anz.*, **97**: 80.

MITOSIS IN AMŒBA DUBIA [1]

J. A. DAWSON, WALTER R. KESSLER, AND JOSEPH K. SILBERSTEIN

(From the Department of Biology, the College of the City of New York)

Since the first accurate description of the two common, large, free-living amœbæ, *Amœba dubia* and *Amœba proteus,* by Schaeffer (1916) very little has appeared in scientific literature concerning the process of mitosis in either of these species. The only reasonably complete account is that of Chalkley and Daniel (1933) in which the nuclear phases of division in *Amœba proteus* are described. Awerinzew's paper (1904) is much too brief and indefinite to give an adequate picture of the process and it is certainly not established that his work was done on *Amœba dubia.* The figures of Carter (1912) are unlike any phase seen by us in this work. Furthermore, certain phenomena observed by the writers have not yet been described in any account previously published concerning division in free-living amœbæ.

The senior author has been interested for a considerable time in specific differences between *Amœba proteus* and *Amœba dubia.* Differences in their structure and physiology have already been noted (Dawson and Belkin, 1929). As the nucleus of *Amœba dubia* is morphologically very different from that of *Amœba proteus,* it was thought that a study of mitosis in the former would reveal still further specific differences.

Material and Methods

The material used was a strain of *Amœba dubia* descended from a single individual which was isolated from a culture collected in a pond in the vicinity of Woods Hole, Mass., in 1926. This strain and others of the same origin have been cultivated continuously by the senior author since that time. An account of the method of culture has been previously published (Dawson, 1928). With but slight modification this method has been used in obtaining dividing animals for this work.

---

[1] The authors wish to express their indebtedness to Dr. James Kendall of the College of the City of New York for his kind assistance in preparing the photomicrographs used in this paper.

It was found relatively simple to obtain cultures in which forty to fifty individuals in all phases of division could be isolated in the course of a single afternoon. The dividing amœbæ were isolated from these cultures by means of a Spencer binocular dissecting microscope using transmitted light.

The organisms to be stained were isolated by means of a capillary pipette of appropriate size and placed on a coverslip with a minimum amount of culture medium. The following fixatives were used: Schaudinn's fluid, strong both with and without acetic acid, Bouin's (the B–15 modification of Allen), strong Flemming's and Carnoy and Lebrun's fluid. Of all these fixatives the one most used was Carnoy and Lebrun's fluid. The picture was essentially the same with the various fixatives. Carnoy and Lebrun's fluid has the great advantage of making the fixed amœbæ adhere to the coverslip, thus greatly facilitating the staining and clearing process. Specimens were usually taken up through the various alcohols and staining and clearing solutions on the coverslip although a number were carried up in the solutions while floating loose in a small culture dish.

The following stains were used: Heidenhain's hematoxylin (Grübler's); gentian violet according to the technique of Johansen (1932). This method is rapid and is particularly useful for comparison of series of preparations. We also used picrocarmine, which is exceptionally good for clarity of detail since it stains chromatin well, giving a good contrast with other structures. The larger achromatic bodies stained very slightly with this stain and the chromatin was a clear red. Delafield's hematoxylin was used occasionally, and although a good general stain, showed no significant features not shown by other stains. Of all these the stain most generally used was Heidenhain's hematoxylin. Both long and short methods of staining were employed. Differentiation was done with iron alum and also in other cases with a saturated aqueous solution of picric acid following the method recommended by Dr. G. W. Kidder (1933). In general, it was found that Heidenhain's hematoxylin gave a clearer picture of mitotic phenomena than any other single stain. Although much more difficult to operate due to danger of loosening the animal, the picric acid-ammonia method of differentiating was found to be superior to the iron alum treatment.

The Feulgen method for differentiation of chromatin was used. Feulgen's procedure as given in McClung's Handbook of Microscopical Technique (p. 483) and the modification presented in Lee's Handbook (p. 306, ninth edition, 1928) were employed. In some cases Feulgen preparations were counterstained with light green. Staining for volutin granules in various stages of the vegetative cycle of *A. dubia* was done. The standard technique for volutin staining was used.

Amœbæ in vegetative phases and in division phases were imbedded in paraffin and sectioned. Sections 4 to 8 μ in thickness were cut. The method of imbedding was similar to that used previously by the senior author (Dawson, 1926). In some cases amœbæ were stained, differentiated and then removed from the coverslip and imbedded and sectioned.

Nuclear Structure of Vegetative Amœba

As described by Schaeffer (1916) the nucleus of *Amœba dubia* is "a prolate spheroid; chromatin distributed in several thousand masses of from one and one half to two microns immediately under the nuclear membrane; size of nucleus about 40 μ by 32 μ short diameter." He makes no mention of the internal structure of the nucleus in this species. In a later paper (1926) the same author classes the nuclei of *Amœba proteus* (*Chaos diffluens*) and *Amœba dubia* (*Polychaos dubia*) under the same general type and states that in this type the chromatin occurs in the living 'resting nucleus' "in a layer of small grains of uniform size at a greater or less distance from the nuclear membrane." The illustrations of this type show invariably a clear space in the central part of the nucleus. Chalkley and Daniel (1933) say that "in the nucleus of *A. proteus* the chromatin, which in the resting nucleus is distributed in granules or 'blocks' immediately beneath the nuclear membrane, leaves this position and moves toward the center of the nucleus." Doflein (1918) describes the chromatin as arranged under the nuclear membrane and also a definite endosome centrally placed in the nucleus of *Amœba proteus.* Taylor (1923) figures the nucleus of *A. proteus* with a karyosome and peripherally arranged chromatin granules under the nuclear membrane. One may conclude from these and other sources that, in the nucleus of *A. proteus,* the chromatin is arranged peripherally under the nuclear membrane and that an endosome (karyosome) may or may not be present.

The nuclear membrane of *Amœba dubia* is clearly defined and can easily be seen in both living and stained preparations. In the stained vegetative nucleus a uniform distribution of staining granules approximating the size of those described by Schaeffer occurs throughout. Our measurements of the staining granules taken from sectioned nuclei give a size range of from 0.2 to 1.7 μ in diameter. In examining a total mount of a vegetative or 'resting' amœba, a peripheral arrangement of staining granules in the nucleus seems to be indicated. This, however, is an optical illusion due to the appearance of these granules in the optical axis. By careful focussing one may determine that the granules are regularly arranged throughout the entire nucleus. That this is a fact is shown clearly by serial sections of the vegetative nucleus (Figs.

1, 21, *a–g*). In the sections the granules do not seem to be connected and the matrix in which they are imbedded is achromatic. No evidence of a fibrillar structure of the matrix was found in any of our sections. Feulgen preparations of vegetative amœbæ were largely negative. In some cases it was possible to detect a slight pinkish color in the granules, presumably chromatin, of the nucleus; this, despite the fact that in the same preparation the nuclei of ciliates gave the typical Feulgen reaction.

The Process of Fission as Seen in the Living Amœba

The various phases of fission in *Amœba dubia* (called by the author *Amœba proteus*), in respect to changes of cytoplasmic form only, have been described by Botsford (1926). There are no outstanding differences of form between Botsford's figures for *A. dubia* and Chalkley and Daniel's for *A. proteus*. We also have found that this is true. Botsford has stated that she found it impossible to observe either nucleus or contractile vacuole while the amœba was in the so-called 'division sphere.' Chalkley and Daniel, on the other hand, noted in *A. proteus* a 'hyaline area' which persisted during the greater part of the division process and only disappeared during the late anaphase. This hyaline area, according to these authors, indicated the position of the nucleus. In the division sphere of *Amœba dubia* we found, as Botsford did, that it was impossible to observe any trace of the nucleus. No hyaline area has ever been seen by us. The earliest stage in fission in *Amœba dubia* shows a general rounding of the periphery and a shortening of the pseudopodia throughout. At this stage the amœba, resting on the glass surface of the container, is slightly thicker than usual. Within a few minutes the organism becomes almost completely spherical—the so-called 'division sphere'—extremely opaque and the pseudopodia become much shorter. We have found that the division sphere persists practically unchanged for a period which varies from fifteen to twenty minutes (Plate I, Fig. 2, *a* and *b*, and Fig. 3). As the division process con-

---

Explanation of Plate I

Fig. 1. *Amœba dubia* Schaeffer. Total mount. × 1350. View of vegetative form showing nuclear structure and two food vacuoles.

Fig. 2. Division spheres in *Amœba dubia* Schaeffer. *A*. Total mount showing anaphase (barrel stage). Note spherical form and relatively short regular pseudopodia. *B*. Total mount showing early telophase. Note similarity of division sphere and pseudopodia. *A* and *B*. × 450.

Fig. 3. *Amœba dubia* Schaeffer. Total mount. × 450. View showing late telophase. Note the connecting strand. The organism is heavily stained due to enormous number of food vacuoles.

Fig. 4. *Amœba dubia* Schaeffer. Total mount. × 450. View showing one method of cytoplasmic division in *A. dubia*. See text.

Fig. 5. *Amœba dubia* Schaeffer. Total mount. × 450. A later view of the same method of division as shown in Fig. 4. Note heavy connecting cytoplasmic strand.

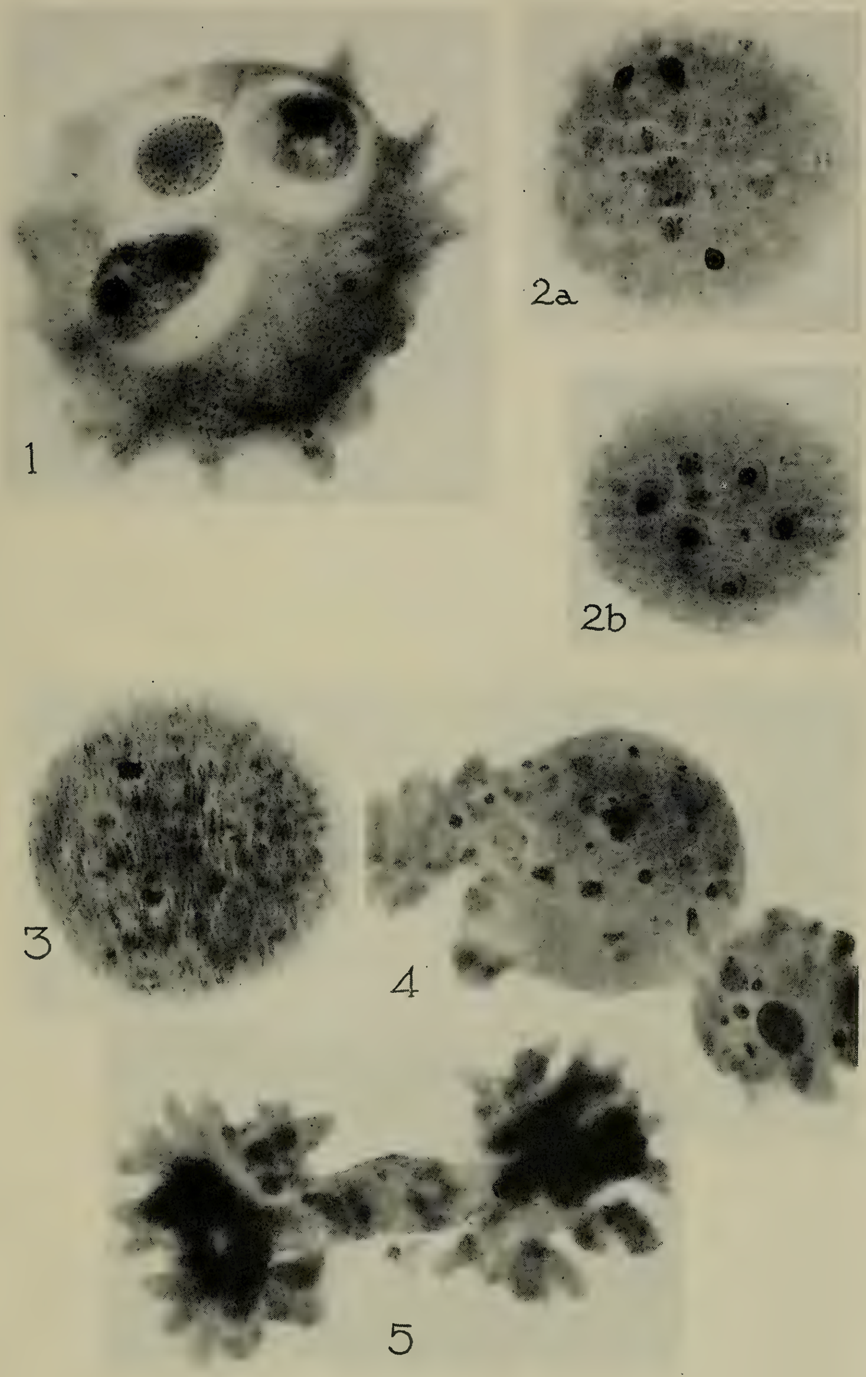


Plate I

tinues, the sphere becomes slightly elongated. This elongation is a definite indication of the late telophase. From here on the external form of the dividing amœba may vary. In about 75 per cent of all cases, short, irregular pseudopodia break out at each pole. The remaining portion of the peripheral protoplasm of the former division sphere (plasmalemma and plasmagel of Mast) remains intact and no pseudopodia are projected from this region. A progressive flowing out occurs at each end and the pseudopodia of each prospective daughter amœba become longer and slightly thinner, still, however, remaining noticeably thickened. The cytoplasmic bridge gradually becomes more attenuated, finally forming a fairly long connecting strand (Plate I, Fig. 5). Cytoplasmic currents, as evidenced by the motion of crystals, can be observed to flow, first in one direction and then in the other in this connecting strand which now appears quite homogeneous and clear. Just before this strand breaks all motion of protoplasm ceases. Each broken end snaps back toward its respective daughter amœba, and, becoming broader and thicker, is gradually withdrawn.

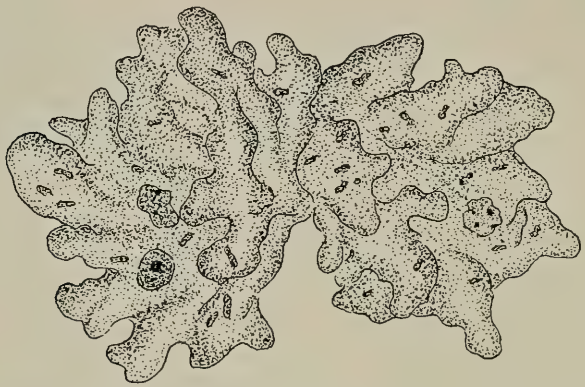


FIG. 6. *Amœba dubia* Schaeffer. Total mount. × 450. A view of the second type of division in *A. dubia*. See text.

In other, fewer cases under apparently similar conditions no long connecting cytoplasmic strand can be seen. Here the pseudopodia are coarser and fewer in number and seem to flow in all directions. Unlike the former type, they are projected from the original central portion which makes the formation of a long connecting strand impossible. Instead, two wedge-shaped areas progressively pass through and cut off the daughter amœbæ (Fig. 6). In such cases the pseudopodia of the daughter amœbæ usually obscure the process of fission and it is only possible to know that it has been completed when the daughter cells move away.

Although the amœba while in the division sphere shows no signs of external motion, a condition of great internal activity prevails. Due largely to the spherical form and to the fact that *Amœba dubia* just before division (*cf*. Fig. 3) may ingest food actively, thus filling itself with many food vacuoles, it has been impossible to see the nucleus during fission. The cytoplasm, however, may be observed. It is in a state of extreme activity. There seems to be no phase difference between endo- and ectoplasm. Crystals and other inclusions are moved actively through the cytoplasm. In this 'boiling' motion the contained bodies appear to be shot against the plasma membrane and to rebound from it.

THE PROPHASE

In *Amœba dubia* the vegetative or 'resting' nucleus consists of a nuclear membrane surrounding several thousand granules uniformly distributed through an achromatic matrix. These granules stain deeply with Heidenhain's hematoxylin and faintly with Feulgen stain. These we consider to contain the chromatin. From the vegetative condition to the prophase a disintegration or breaking down of the chromatin granules occurs. The earliest division phase shows a uniform, lightly staining matrix in which no fibers are evident. In this phase there now appear, peripherally arranged, a considerable number of larger achromatic bodies. The size of these varies from 0.5 μ to 4.0 μ and in the earliest phase we have counted as many as 50 in a single nucleus (Figs. 8 and 27). We have not, however, been able to determine with certainty the origin of these bodies.

In the later prophase, fibers may be seen. These fibers at first seem to run somewhat obliquely to the larger equatorial plane of the nucleus. The nuclear membrane remains intact and in appearance does not differ from the condition found in the vegetative nucleus. The cytoplasm immediately surrounding the nucleus is quite clear in all preparations. No difference can be detected in its appearance at this stage and in the 'resting' condition. The prophase lasts for approximately five minutes at a room temperature of 22° C. In this respect it is quite similar to the prophase of *A. proteus* as determined by Chalkley and Daniel. Due to lack of specific indicative features, it is difficult to obtain other than approximate time for this phase.

METAPHASE

Although several preparations of the early metaphase were obtained, no definite equatorial plate as shown by Chalkley and Daniel for *A. proteus* could be observed. This we believe to be due to the fact that such a plate obviously lasts for but a very short time interval. At this time also the chromatin stains very faintly. The nuclear membrane remains intact during this phase. In this respect *A. dubia* differs markedly from *A. proteus* in which according to Doflein (1913) and Chalkley and Daniel (1933) the nuclear membrane breaks down. The nucleus now takes a different shape, becoming elongated along the line of its shorter axis. The peripheral achromatic bodies begin to assume positions at the slightly invaginated poles. The spindle fibers fill the nucleus completely although they appear somewhat fainter in the polar regions (Fig. 28). The individual fibers are extremely fine, the number easily exceeds one hundred.

In the late metaphase the chromatin (chromosomes) now forms two well marked plates approximately 2.5 to 3.5 μ in thickness. The chromosomes are so numerous and small that we have been unable to identify single individuals.

ANAPHASE

In the early anaphase the nuclear form may best be described by calling it a 'barrel.' The spindle fibers fill the barrel running from top to bottom. The outer surface of the barrel is the nuclear membrane (Figs. 2*a*, 9, 10, 23, 29, and 30) which is slightly depressed or wrinkled

---

EXPLANATION OF PLATE II

Photomicrographs. All figures were photographed using a Leitz compound microscope and Leitz apochromatic lenses. Oil immersion lens, N.A. 1.32 and 15 × ocular were used in all cases except in Fig. 7 which was taken with a 5 mm. lens. Figures 11 and 17 were stained with Gentian Violet. Figure 12 with Picrocarmine. All others were stained with Heidenhain's hematoxylin. All figures are magnified 1350 diameters except Fig. 7.

FIG. 7. Vegetative nucleus showing dispersed chromatin granules. × 450.

FIG. 8. Prophase. Note large achromatic bodies and lack of chromatin granules. Same specimen drawn in Fig. 27.

FIG. 9. Anaphase (early barrel stage). Completely formed achromatic figure. The same nucleus is drawn in Fig. 29.

FIG. 10. Slightly later stage of anaphase. The same figure is drawn in Fig. 30.

FIG. 11. Late anaphase. The further separation of the chromatin plates is shown. Note also polar aggregation of achromatic bodies.

FIG. 12. Early telophase. Compare with Fig. 31.

FIG. 13. *a.* Stage similar to that in Fig. 12. The nucleus to the right is in sharper focus. Note chromatin plate. *b.* Same specimen showing left nucleus and spindle in sharp focus. Note numerous spindle fibers.

FIG. 14. Slightly later telophase.

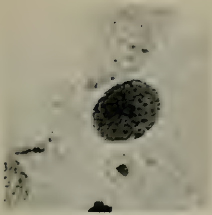
FIG. 15. Telophase. Note beginning of 'dumb-bell' appearance and hemispherical daughter nuclei.

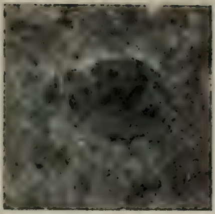
FIGS. 16 and 17. Telophases showing further separation of prospective daughter nuclei. Note twisting of spindle. Hyaline polar zones shown in Fig. 17.

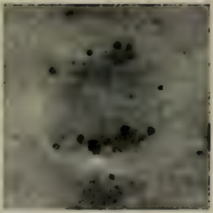
FIG. 18. Telophase showing daughter nuclei in two views.

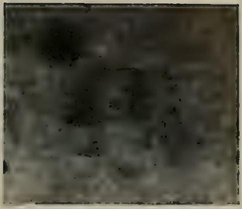
FIG. 19. Late telophase showing 'thread' and ameboid daughter nuclei.

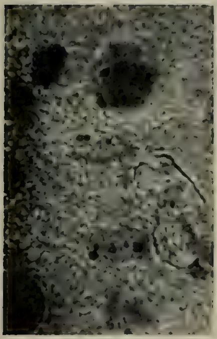
FIG. 20. Similar to Fig. 19 but somewhat later stage.

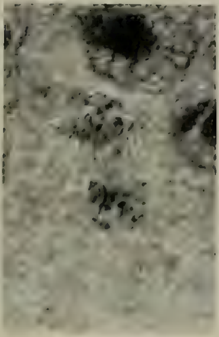

7


8

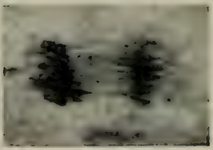

9


10

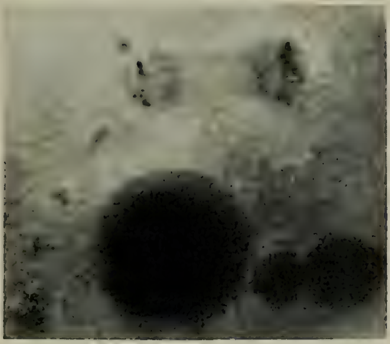

11

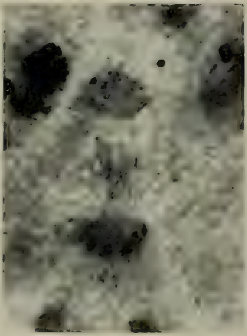

12

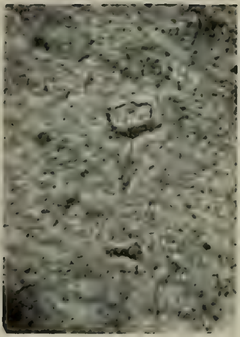
13a


13b

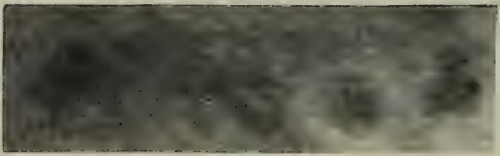
14


15


16


17

18


19

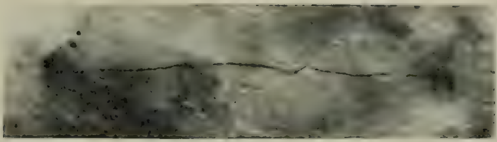

20

PLATE II

at each pole. From earlier to later stages of this phase the distance between the chromatin plates increases. In the late anaphase the majority of the peripheral achromatic bodies have assumed a polar position, i.e., between the chromatin plate and the pole.

TELOPHASE

In the early telophase the barrel shape changes into that of a ' dumb-bell ' with a very thick handle. The somewhat flat and more or less indented polar regions of the barrel now assume a hemispherical form, still containing practically all of the achromatic bodies. The spindle fibers extend from pole to pole and, in some preparations, appear to pass through the nuclear membrane which is attenuated in these regions. The chromatin plates occupy a position at each end of the handle of the dumb-bell, i.e., where the handle joins each rounded part (Figs. *2b*, 11, 12, 13, 14, 15, 16, 31, and 32).

As the telophase progresses the prospective daughter nuclei gradually move farther apart and the distal portion of each appears distinctly hyaline. The achromatic bodies, which now have clearly decreased in number, are aggregated in these hyaline zones (Figs. 17, 18, and 33). The chromatin plates which in the early telophase were clearly defined now begin to lose this character and become dispersed through each daughter nucleus, obliterating the hyaline areas in each. With the further separation of the daughter nuclei, the spindle fibers become noticeably fewer in number. Almost invariably a twisting of the spindle cylinder occurs at this stage (Figs. 16, 17, and 32).

In the late telophase the outstanding feature is the transition of the elongated, twisted spindle cylinder to a long, thick, deeply-staining

---

EXPLANATION OF PLATE III

Photomicrographs. All magnifications × 1350. With the exception of Figs. 26 and 27 all figures are from sectioned amœbæ. Sections 5 μ thick. Fig. 25 from specimen stained with picrocarmine, all others stained with Heidenhain's hematoxylin.

FIG. 21, *a–g*. Views of serial sections, complete, of a typical ' resting ' nucleus of *Amœba dubia*. Fig. 21 shows the entire section of the amœba, the nucleus of which is again represented in Fig. 21*e*. Note the uniform distribution of the chromatin granules in all sections of the nucleus.

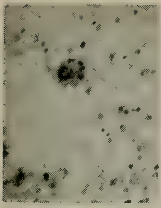
FIG. 22 *a, b,* and *c*. Successive sections through an early metaphase. Sectioned obliquely.

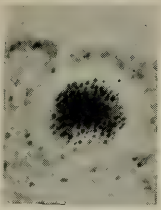
FIG. 23. Late anaphase. Median longitudinal section. Note achromatic figure.

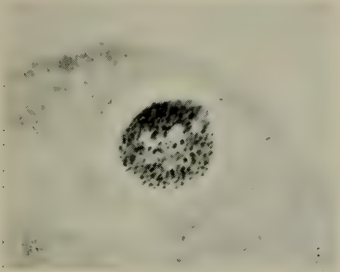
FIG. 24. Late telophase showing ameboid nucleus and part of thread. Nucleus staining heavily in this stage.

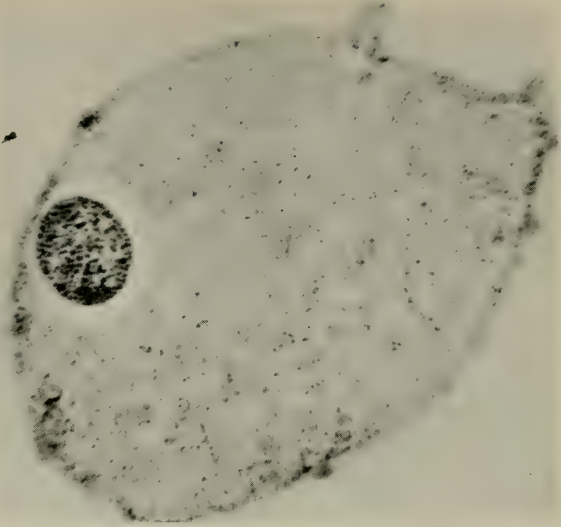
FIG. 25. Total mount. Reconstructing nucleus soon after fission. Note presence of large achromatic bodies and reappearing chromatin granules.

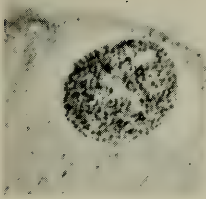
FIG. 26. Reconstructing nucleus about two and one half hours after fission. Same nucleus as drawn in Fig. 36.

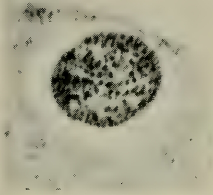

21a

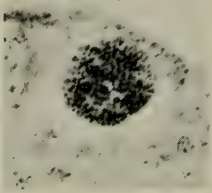

21b

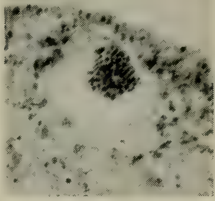

21c

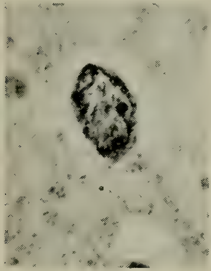

21

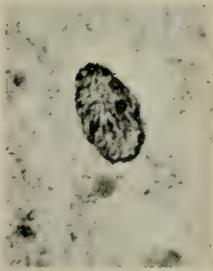

21d

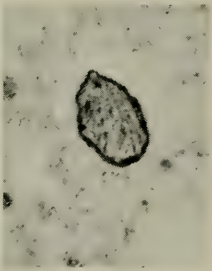

21e

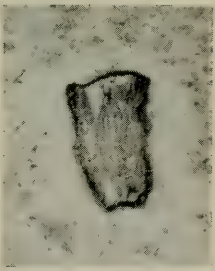

21f

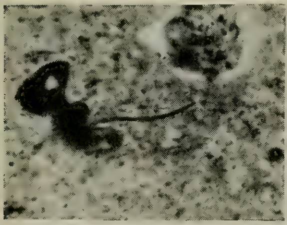

21g

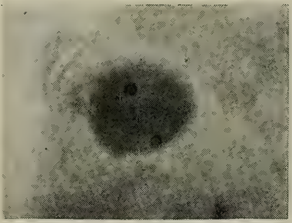

22a


22b


22c


23


24


25

26

Plate III

strand or thread. That the major part of this strand is formed by the nuclear membrane is impossible to doubt since it can be followed completely around each daughter nucleus. At the place where the strand passes into the nucleus, a conical, vacuolated region may be seen (Fig. 34). The form of each daughter nucleus gradually changes from the roughly hemispherical condition in the dumb-bell stage to that of one which is quite ameboid in appearance (Figs. 19, 20, 24, 33, and 34). The daughter nuclei occupy a position at opposite ends of the now elongated mother amœba. They are extremely close to the periphery. Each daughter nucleus now stains much more heavily throughout. The chromatin appears as very small, staining granules. These granules increase rapidly in size and are quite noticeable at the moment of fission. They are quite uniform in size, measuring approximately 0.2 μ in diameter. The telophase nuclei show a striking contrast in apparent size. This is due to the fact that one of the nuclei is so oriented as to show a surface view while the other is seen from the side (Fig. 18). The conspicuous fiber formed from the nuclear membrane breaks, so far as ascertained, apparently shortly after the cytoplasmic separation has begun. In some cases we have found that this break occurs close to one of the nuclei. We have no evidence that it is reabsorbed since no stages in that process have been found in many preparations made at

---

EXPLANATION OF PLATE IV

All drawings made with camera lucida. × 2100.

FIG. 27. Prophase (same as Fig. 8). Chromatin granules of vegetative nucleus have disintegrated. Large achromatic bodies clearly defined in lightly staining nuclear matrix. Note slight invaginations at poles.

FIG. 28. Slightly later stage. Formation of achromatic figure is completed. Slight elongation of nucleus has taken place and polar invaginations are more prominent.

FIG. 29. Late metaphase. Illustrates typical 'barrel' stage. Note chromatin plates staining faintly and polar aggregation of achromatic bodies. Nuclear membrane clearly marked (same as Fig. 9).

FIG. 30. Anaphase (late). Here chromatin plates stain more heavily and the distance between the plates has increased (same as Fig. 10).

FIG. 31. Early telophase. Note beginning of 'dumb-bell' with thick handle, and hemispherical form of polar regions containing most of the achromatic bodies (same as Fig. 12).

FIG. 32. Later telophase. Chromatin plates are not so pronounced. Typical twisting of spindle cylinder shown (same as Fig. 16).

FIG. 33. Still later telophase. Reduction in number of spindle fibers. Daughter nuclei assuming typical ameboid shape. Reduction in number of achromatic granules. Entire nucleus staining more deeply.

FIG. 34. Latest telophase. Note characteristic 'thread.'

FIG. 35. Showing nucleus just after fission is completed. Note presence of numerous fine granules and dispersion of achromatic bodies throughout the ameboid nucleus.

FIG. 36. Reconstructing nucleus two and a half hours after fission. Note increase in size of nucleus and of chromatin granules. Achromatic bodies still present. Nucleus more regular in outline (same as Fig. 26).

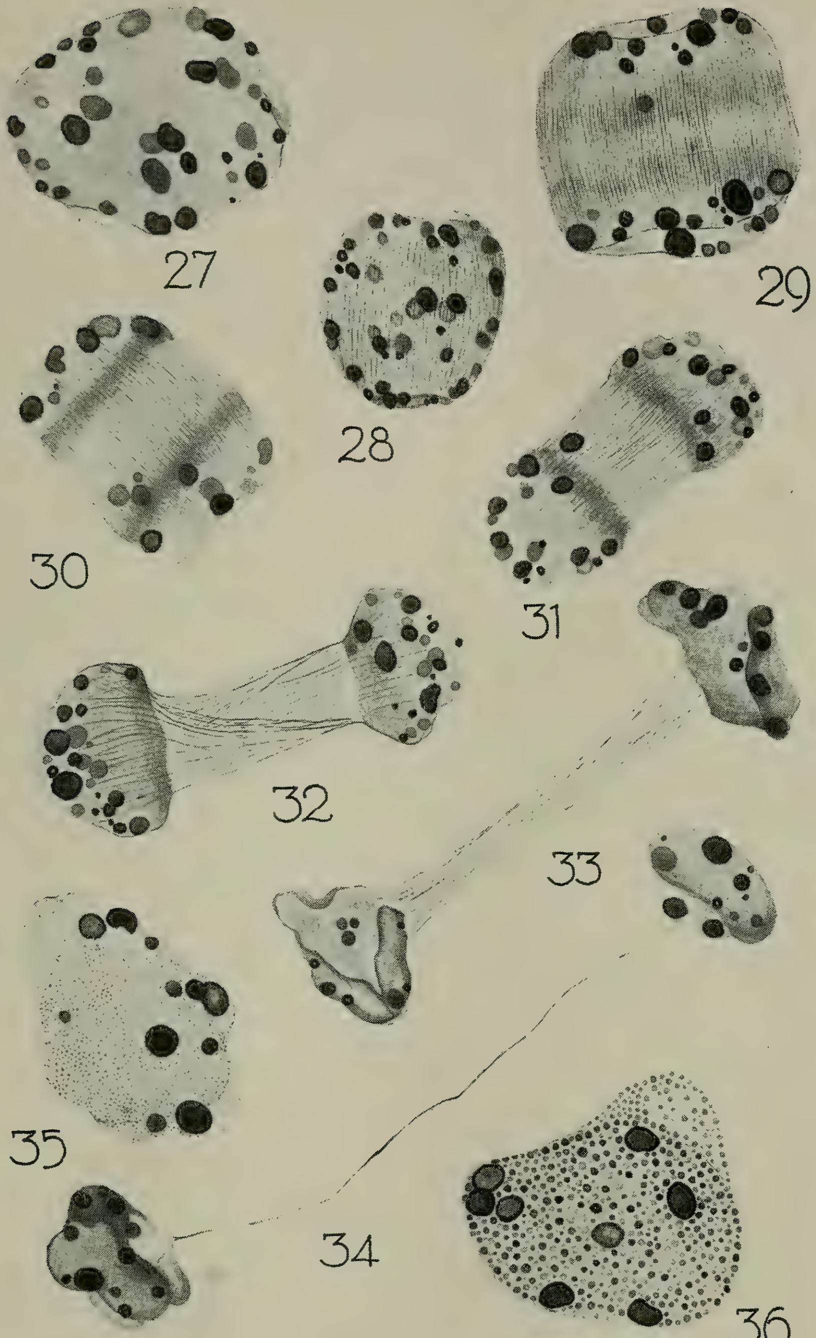

27
29
28
30
31
32
33
35
34
36

PLATE IV

this time. No remnants of it ever appear in newly divided daughter cells. We infer that it is resorbed by the cytoplasm.

RECONSTRUCTING NUCLEI

The characteristic appearance of the newly separated nucleus is like that of a small amœba with very blunt pseudopodia. This nuclear configuration not only appears in all stained preparations of this stage but also may be seen in the newly divided daughter amœbæ (Fig. 35). In the early reconstructing nucleus the achromatic bodies are present but few in number (Figs. 25 and 35). A few minutes after division the chromatin is present in the form of very numerous, staining granules slightly smaller than those found in the typical resting nucleus. The picture shown by the reconstructing nucleus is as follows—within a varying period (three to five hours) the outline of the nucleus becomes regular like that of the ellipsoidal vegetative nucleus. During this time the achromatic bodies gradually disappear. They vary considerably in size up to their final disappearance in the typical resting nucleus. Figures 26 and 36 show the same reconstructing nucleus two and a half hours after fission. At this time the size of the chromatin granules is $0.8\,\mu$ in diameter.

DISCUSSION

In the present study of mitotic division in *Amœba dubia* a number of significant differences between this species and other free-living amœbæ have been found. We believe that these variations depend upon fundamental structural differences in the nucleus. It has already been shown that the nucleus of *Amœba dubia* does not conform with any of the types of nuclei described by Schaeffer (1926) in his comprehensive review of the biology of amœba. In complete studies of the process by numerous investigators, e.g., Gläser (1912), Dobell (1914), Wasielewski and Kühn (1914), Jollos (1917), Doflein (1912) and others, the nucleus is invariably described as possessing an endosome or karyosome. In the nucleus of *Amœba dubia* no endosome exists but the chromatin is evenly distributed throughout the entire ‘ resting ’ nucleus.

One of the outstanding features is that the nuclear membrane remains intact throughout the entire process. In the closely related species, *A. proteus* (Chalkley and Daniel, 1933) the nuclear membrane entirely disappears during the early anaphase. In general this is in accord with the findings of the above-mentioned investigators with the exception of Gläser. It would seem clear from our study that both chromatic and achromatic elements of the spindle are derived solely from the nucleus.

A second interesting feature is the presence of the large achromatic bodies to which we have so often referred. In our preparations we have used a considerable variety of both fixatives and stains. These bodies do not stain with any chromatin stains. As previously stated, these appear in large numbers in the early prophase, they are present throughout the entire process of fission and gradually disappear as the nucleus is reconstructing. There may be a correlation between the disappearance of these achromatic bodies and the increase in size of the chromatin granules in such nuclei. At present we are unable to offer an explanation of the constitution and function of these bodies.

The mitotic process in *Amœba dubia* as shown by this study shows striking differences to that of *Amœba proteus* and thus supports the view that these two common, large amœbæ are quite distinct species.

LITERATURE CITED

AWERINZEW, S., 1904. Über die Teilung bei Amœba proteus Pall. *Zool Anz.,* **27**: 399.

BOTSFORD, E. FRANCES, 1926. Studies on the contractile vacuole of Amœba proteus. *Jour. Exper. Zoöl.,* **45**: 95.

CARTER, LUCY A., 1912. Note on a case of mitotic division in Amœba proteus Pall. *Proc. Roy. Phys. Soc. Edin.,* **19**: 54.

CHALKLEY, H. W., AND GEORGE E. DANIEL, 1933. The relation between the form of the living cell and nuclear phases of division in Amœba proteus (Leidy). *Physiol. Zoöl.,* **6**: 592.

DAWSON, J. A., 1926. A mutation in Paramecium aurelia. *Jour. Exper. Zoöl.,* **44**: 133.

DAWSON, J. A., 1928. The culture of large free-living Amœbæ. *Am. Nat.,* **62**: 453.

DAWSON, J. A., AND MORRIS BELKIN, 1929. The digestion of oils by Amœba proteus. *Biol. Bull.,* **56**: 80.

DOBELL, C., 1914. Cytological studies on three species of Amœba. *Arch. für Protist.,* **34**: 139.

DOFLEIN, F., 1918. Die vegetative Fortpflanzung von Amœba proteus Pall. *Zool. Anz.,* **49**: 257.

GLÄSER, HANS, 1912. Untersuchungen über die Teilung einiger Amöben, zugleich ein Beitrag zur Phylogenie des Centrosoms. *Arch. für Protist.,* **25**: 27.

JOHANSEN, DONALD A., 1932. A new method of differentiating gentian violet when used as a somatic chromosome stain. *Stain Tech.,* **7**: 17.

JOLLOS, VICTOR, 1917. Untersuchungen zur Morphologie der Amöbenteilung. *Arch. für Protist.,* **37**: 229.

KIDDER, GEORGE W., 1933. Studies on Conchophthirius mytili de Morgan. I. Morphology and division. *Arch. für Protist.,* **79**: 1.

SCHAEFFER, ASA A., 1916. Notes on the specific and other characters of Amœba proteus Pall. (Leidy), A. discoides spec. nov., and A. dubia spec. nov. *Arch. für Protist.,* **37**: 204.

SCHAEFFER, ASA A., 1926. Recent discoveries in the biology of Amœba. *Quart. Rev. Biol.,* **1**: 95.

TAYLOR, MONICA, 1923. Nuclear divisions in Amœba proteus. *Quart. Jour. Mic. Sci.,* **67**: 39.

WASIELEWSKI, TH., AND A. KÜHN., 1914. Untersuchungen über Bau and Teilung des Amöbenkerns. *Zool. Jahr. Anat.,* **38**: 253.

THE CHROMOSOME CYCLE OF PHENACOCCUS (COCCIDÆ)

SALLY HUGHES-SCHRADER

(From the Department of Zoölogy, Columbia University)

Within the insect family Coccidæ are species in which male haploidy is completely established and others in which a physiological haploidy seems to be in progress of evolution through the inactivation of one of the haploid sets of chromosomes in the male line. To the latter group belongs *Phenacoccus acericola.* In general its cytology conforms closely to the *Pseudococcus* type (F. Schrader, 1921, 1923) ; but the final stages of spermatogenesis are fortunately more open to analysis and the complete loss of those spermatid nuclei which receive the degenerate haploid contingent of chromosomes can here be established without a doubt. The case is thus also closely allied to that of *Gossyparia* (F. Schrader, 1929), but it is not complicated by the peculiar pairing among the degenerate chromosomes which partially obscures the interpretation of events in the latter species. These general conclusions on *Phenacoccus* were tentatively expressed in a review of coccid cytology in relation to haploidy (F. Schrader and Hughes-Schrader, 1931) ; the data demonstrating them are now complete and it seems wise to present the proof in brief form in order to complete the record.

General Biological Features

The sugar maple is the only known host of *Phenacoccus acericola.* Infections are sporadic and of rather rare occurrence. My material comes from a small colony on a single tree in the Arnold Arboretum, Boston, and a larger colony infesting a group of trees in the depot park at Wellesley, Massachusetts. I am indebted to Dr. Harold Morrison of the U. S. Bureau of Entomology for the discovery and identification of the first colony, and to Dr. M. A. Hayden of Wellesley College for continued assistance in connection with the latter.

Only a single generation is produced annually. Adult males appear late in August and early September. The fertilized females hibernate in crevices of the bark, emerging early the following summer and attaching themselves to the main veins on the under side of the leaves. The eggs are laid in a loose wax mass adherent to the body of the female;

on hatching the larvæ crawl out and attach to the smaller veins of the leaves. At the start of the third instar the male larvæ spin characteristic wax cocoons in which they complete the two final molts. These cocoons are usually hidden away in twig axils or bark crevices.

Meiosis in the male occurs in the third instar, with spermatid development carrying over into the fourth. The eggs develop slowly throughout the summer, maturation and fertilization occurring during the early fourth instar. The eggs are fertilized in the oviducts and development proceeds to varying stages of embryology before the eggs are laid. No evidence of parthenogenesis was found, but this has not been checked by adequate breeding experiments.

Best fixation was obtained with Allen's modification of Bouin's fluid, after a complete dissection of ovaries and testes in the fixative. The eggs must be punctured to insure penetration. Ovaries were sectioned at 5 and 6 micra, testes at 3 micra,—as the cells are extremely small. The preparations illustrated were all stained with the Haidenhain iron hematoxylin method.

Cytological Observations

The diploid chromosome number in both sexes is twelve. The cytology of the female is normal in every respect. The eggs undergo two maturation divisions with typical synapsis, tetrad formation, and reduction of the chromosomes to the haploid set of six.

As indicated above, the interest of the present case lies in the chromosome cycle of the male line. Here one whole haploid group of chromosomes maintains a permanently condensed form, massing into a pycnotic knot during the resting phases, and separating into its component chromosomal elements for each division. Somatic and spermatogonial cells all show this permanent pycnosis of one chromosome set.

The prophase of the primary spermatocyte is initiated by the gradual separation of the pycnotic mass into its six component chromosomes (Figs. 1, 2, and 3). Only then do the normal chromosomes emerge from the chromatin reticulum as fine threads which shorten, thicken, develop each an equational split (Figs. 4 and 5), and finally condense into the small rod-shaped chromosomes of the metaphase stage (Fig. 6). The permanently condensed group is distinguishable for some time by its close aggregation, the normal chromosomes usually surrounding those of pycnotic origin rather loosely (Fig. 6); with the completion of the metaphase plate, however, this distinction is lost (Fig. 7). The first division proceeds as a normal mitosis; a typical bipolar spindle is formed and each chromosome divides equationally (Figs. 8 and 9). Early in the anaphasic movement the chromosomes of each daughter group segregate once more into two groups; one set of six adheres closely together, often in ring formation, and retains the compact form and densely staining aspect of the metaphase chromosomes; the other six show no tendency to aggregate and stain more lightly. In Figs. 10, 11, and 12 the ring-shaped group of six is seen from its side; the polar aspect of the telophase group (Fig. 13) shows a top view of the ring, surrounded by the six loosely arranged chromosomes. No ordinary interphase occurs; the telophase configuration of the first division becomes the metaphase for the second with no rearrangement in position nor change in form of the constituent chromosomes.

From the ring of six condensed chromosomes a half spindle now forms, converging in a single centriole (Fig. 14). The normal chromosomes show no trace of division apparatus other than such an occasional weft or fibril as is shown on one chromosome in Fig. 15. Indeed, from their increasingly indefinite outline, the chromosomes of the normal

---

Plate I

Drawings with Abbé camera lucida, at table level; Zeiss 2 mm. obj. and 20 × ocular.

Fig. 1. Resting phase of pre-meiotic spermatogonium; permanently condensed chromosomes in pycnotic knot.

Figs. 2 and 3. Prophases of primary spermatocytes with condensed chromosomes separating from knot.

Fig. 4. Normal chromosomes evolving as threads from the reticulum in later prophase I.

Fig. 5. Later stage of prophase I. Normal haploid set of chromosomes showing equational split.

Fig. 6. Metaphase I with two haploid groups still spatially distinct.

Fig. 7. Later metaphase I; no distinction between the haploid groups.

Figs. 8 and 9. Lateral views of anaphase I.

Figs. 10, 11, and 12. Later anaphases of first division. Cytoplasmic stalks visible. In each anaphase group the six chromosomes derived from the condensed set form a ring, here viewed from its side.

Fig. 13. Polar view of telophase I, with the two sets of chromosomes distinguishable.

Figs. 14 and 15. Formation of a single half spindle in connection with the condensed chromosome group. Normal group passive.

Figs. 16 and 17. Anaphasic movement of the condensed group to the single pole. Interzonal body forms between the two groups.

Fig. 18. Telophase II, with six chromosomes in each group. No cytoplasmic division.

Fig. 19. Binucleate spermatids; growth of the normal nucleus.

Fig. 20. Fusion of spermatids in pairs, forming quadrinucleate spermatids, each with two normal and two abnormal nuclei.

Figs. 21, 22, 23, 24, and 25. Development of sperm from the normal nuclei; gradual degeneration of the abnormal nuclei.

Fig. 26. The abnormal nuclei reduced to pycnotic knots and sloughed off with the cytoplasmic residue of the cells. Mature sperm forming bundle at edge of cyst.

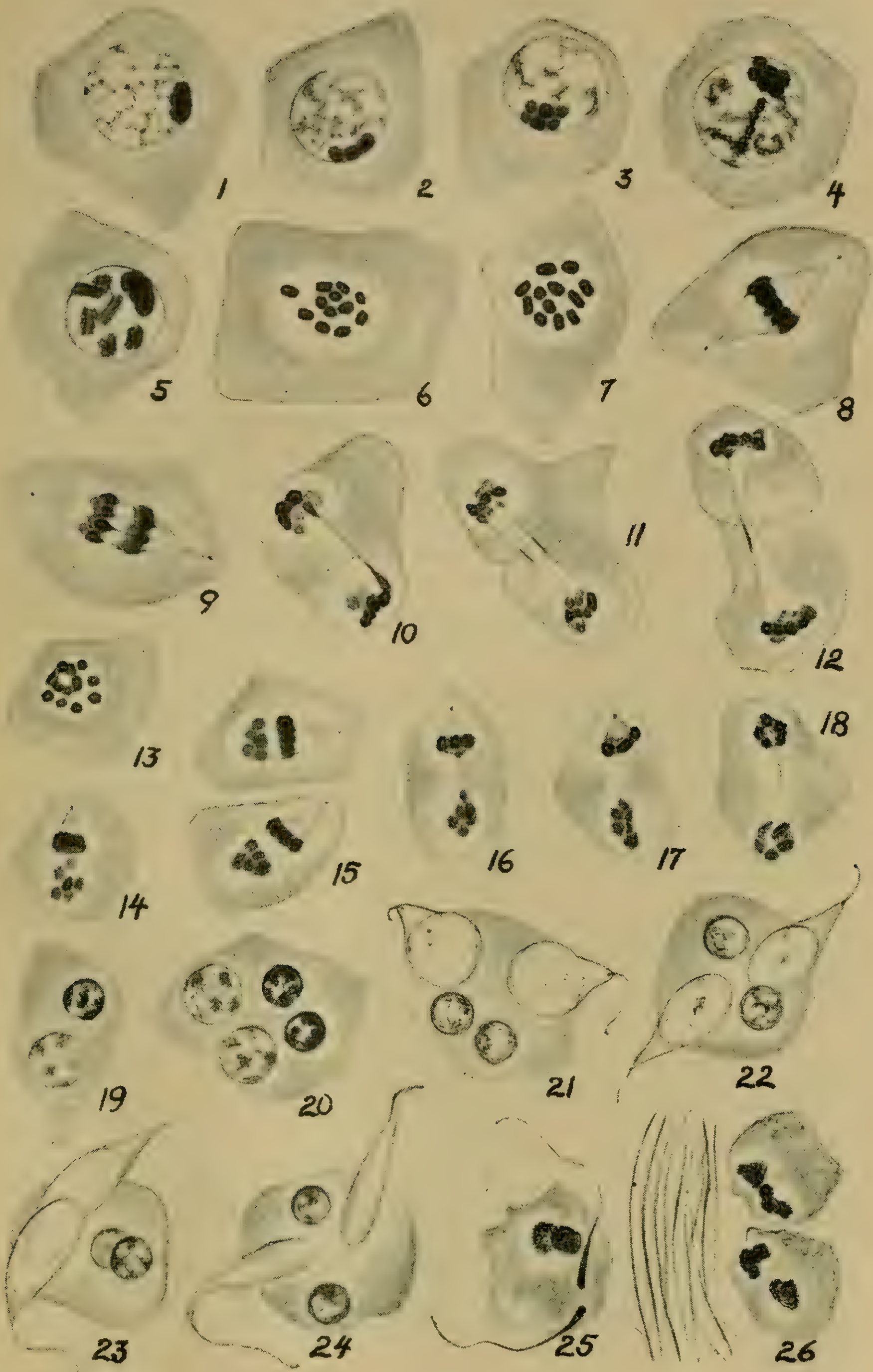

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26

PLATE I

group would seem to be entering an interphasic rest. The condensed group now moves poleward, away from the loose group,—its half spindle shortening and the cell as a whole elongating in the process (Figs. 16 and 17). Between the anaphasic groups a definite interzonal body forms, quite similar in form and staining reaction to those of orthodox mitoses. For a short time the chromosomes are countable at both poles—six in the massed, compact group, six in the looser group. They quickly assume the resting condition and nuclear membranes form around them. No trace of a cytoplasmic division occurs, and all the spermatids are thus at first binucleate.

The binucleate spermatids now fuse in pairs. Some direct observational evidence for this lies in the frequently lobed form of the early quadrinucleate spermatids resulting from the fusion. It seems probable that the first division, although apparently complete cytoplasmically, does not cut through the short centrally directed stalk which each cell of a cyst shows when cut radially (Figs. 9, 10, 11, and 18). The spindle of the first division lies always at right angles to this stalk; thus a division of the cell, apparently complete when viewed from the outside of the cyst, would not cut this stalk (Fig. 12). The refusion of the cells may well proceed from these persistent stalks. A further check on the fusion of the spermatids is available in counts of the number of nuclei per cyst in the affected stages—a method which has proved useful in the study of several other coccids. Cysts of primary spermatocytes contain typically sixteen cells; those with secondary spermatocytes thirty-two. The binucleate spermatids show the same thirty-two cells, but sixty-four nuclei per cyst. After fusion, the cysts of quadrinucleate spermatids contain the sixty-four nuclei expected.[1]

The further history of the spermatid development can be read directly from Figs. 19 to 25 without much comment. The two small darkly-staining nuclei derived from the condensed group of chromosomes show almost no growth, and gradually degenerate *in situ* into crumpled pycnotic knots. From the two more slowly evolving, normal nuclei in each spermatid typical coccid sperm develop (Figs. 19 to 24). The sperm formed in each cyst then coil into a spiral bundle; cross sections of these bundles, although too small and closely packed for accurate counts, show approximately thirty-two sperm per bundle.[1] The degenerate nuclei, sloughed off by the sperm with the cytoplasmic residue of the cell (Fig. 25), are also countable for some time; again the number per cyst is consistently thirty-two,[2] confirming the complete loss of that half of the spermatid nuclei derived from the pycnotic chromosome set.

---

[1] The following counts establish the uniformity of the number of nuclei per cyst, and give evidence of the fusion in pairs of the spermatids.

Number of nuclei per cyst of primary spermatocytes: 16—16—12—16—16—16—3—16—15—16—16—16.

Number of nuclei per cyst of secondary spermatocytes: 32—32—32—31—31—32—27—32—31—24.

Number of nuclei per cyst of binucleate spermatids: 64—64—62—65—61—64—65—68—44—25—48.

Number of nuclei per cyst of quadrinucleate spermatids: 64—62—57—67.

Number of sperm per cyst bundle: 32—33—28—31—34—31—30—28—28—26.

Number of pycnotic nuclei per cyst after sperm formation: 32—31—32.

The degenerate condition of one of the haploid chromosome sets in the male expresses itself somatically as well as in the meiosis of the germ cells. In all the body cells of males the affected chromosomes retain the condensed form throughout the resting phases, mass into a pycnotic knot, and separate into their component elements only at each nuclear division. As in *Gossyparia,* this behavior first becomes visible in young embryos of the blastula stage.

SUMMARY

1. The diploid chromosome number of *Phenacoccus acericola* is twelve in both sexes.

2. The female is normal in all respects. All eggs undergo two maturation divisions with typical synapsis and reduction of the chromosomes to the haploid condition.

3. In the male, from the blastula stage of development on, one haploid set of chromosomes maintains a permanently condensed condition, forming a pycnotic knot in resting phases and separating into its component chromosomes at division.

4. Meiosis in the male is effected without synapsis. The first division is equational and diploid. Without interphase the telophase chromosomes separate into two groups of six, one condensed and closely aggregated, and one loosely arranged and more diffuse. A single half spindle forms in connection with the condensed group and reduction is effected by the consequent passage of the condensed group to the single pole of the spindle.

5. No cytoplasmic division accompanies the second division; the binucleate spermatids thus formed fuse in pairs.

6. That half of the spermatid nuclei originating from the condensed chromosome set disintegrates without trace of sperm development; the other half of the spermatid nuclei, those originating from the non-pycnotic normal chromosomes, develops into typical coccid sperm.

---

[2] See footnote 1.

LITERATURE CITED

SCHRADER, F., 1921. The chromosomes of Pseudococcus nipæ. *Biol. Bull.,* **40:** 259.

SCHRADER, F., 1923. A study of the chromosomes in three species of Pseudococcus. *Arch. f. Zellforsch.,* **17:** 45.

SCHRADER, F., 1929. Experimental and cytological investigations of the life-cycle of Gossyparia spuria (Coccidæ) and their bearing on the problem of haploidy in males. *Zeitschrift f. wiss. Zool.,* **134:** 149.

SCHRADER, F., AND SALLY HUGHES-SCHRADER, 1931. Haploidy in Metazoa. *Quart. Rev. Biol.,* **6:** 411.

INDEX

ACCLIMATIZATION, to high and low temperatures, effect on rate of respiratory metabolism in teleost fish, 361.
Activation, and acid formation in frozen and thawed Arbacia punctulata eggs, 345.
——, influence of iodoacetate on, in Arbacia punctulata eggs, 351.
AMBERSON, WILLIAM R. *See* Bosworth and Amberson (abstract), 339.
——, —— —. *See* Stanbury and Amberson (abstract), 339.
Amoeba dubia, mitosis in, 447.
Anesthetics, susceptibility to, and respiratory metabolism in fishes, 368.
Antuitrin-S, effects on male genital organs of lizard during seasonal atrophy, 143.
Annulus ventralis in true intersexes, Cambarus, 269.
Arbacia punctulata eggs, frozen and thawed, acid formation in, 345.
—— —— —, influence of iodoacetate on activation, 351.
Autotomy, induced, regeneration following, in Thyone, 82.
Avena, phototropic responses in relation to intensity and wave-length, 305.

BACHEM, A., AND M. A. DUSHKIN. A study of bacterial sensitivity to ultra-violet radiation, 109.
BARNES, T. CUNLIFFE. Salt requirements and orientation of Ligia in Bermuda, III, 259.
Blood, chloride and total osmotic pressure, marine teleosts, 245.
BOSWORTH, MILLARD W., AND WILLIAM R. AMBERSON. An adaptation of the manometric Van Slyke apparatus to the study of the respiration of marine animals (abstract), 339.
BOZLER, EMIL. Mechanical properties of smooth muscle (abstract), 343.
BRAND, THEODOR V. Methods for the determination of nitrogen and carbon in small amounts of plankton, 221.
BUDINGTON, R. A. The tolerance of acetyl-salicylic acid by sperm and eggs of Arbacia (abstract), 335.

CAMBARUS, annulus ventralis, in true intersexes, 269.
Carbon, determination, in plankton, 221.
Centrifugal force, influence on cleavage plane and mitotic figure of Parechinus egg, 287.
—— ——, influence on surface phenomena in fertilized sea urchin egg, 298.
Centrifuged eggs of Ilyanassa, formation of giant polar bodies, 403.
Centrioles of Pseudotrichonympha and their rôle in mitosis, 46.
CHAMBERS, ROBERT. *See* Hibbard and Chambers (abstract), 332.
——, ——. Studies on the physical properties of the plasma membrane (abstract), 331.
CHASE, H. Y. The effect of temperature on the rate of the fertilization reaction in various marine ova, 415.
——, —. —. The origin and nature of the fertilization membrane in various marine ova, 159.
Chloride and total osmotic pressure in blood of marine teleosts, 245.
Chromosome cycle of Phenacoccus, 462.
—— structure, in salivary gland cells in Sciara, 126.
CHURNEY, LEON. The quantitative determination of mitotic elongation (abstract), 333.
CLARK, LEONARD B. Dark adaptation in the insect Dineutes assimilis (abstract), 329.
Cleavage plane, egg of Parechinus, as influenced by centrifugal force, 287.
CLEMENT, ANTHONY C. The formation of giant polar bodies in centrifuged eggs of Ilyanassa, 403.

CLEVELAND, L. R. The centrioles of Pseudotrichonympha and their rôle in mitosis, 46.

——, —. —. The intranuclear achromatic figure of Oxymonas grandis, sp. nov., 54.

CLOWES, G. H. A. *See* Krahl and Clowes (abstract), 340.

——, —. —. —., M. E. KRAHL, AND A. K. KELTCH. Stimulation and depression of respiration in relation to cell division (abstract), 341.

COLE, ELBERT C. Methylene blue staining sequences in the walls of the digestive tube of the frog, Rana grylio (abstract), 335.

COPELAND, MANTON. Keeping Nereis for physiological study (abstract), 337.

COSTELLO, DONALD P. The effects of centrifuging the eggs of nudibranchs (abstract), 326.

DAUGHERTY, KATHRYN. *See* Heilbrunn and Daugherty, 279.

DAWSON, J. A., WALTER A. KESSLER, AND JOSEPH A. SILBERSTEIN. Mitosis in Amoeba dubia, 447.

Development, influence of iodoacetate on, in Arbacia punctulata egg, 351.

Differential threshold of reaction to vitamin D deficiency in house sparrow and chick, 71.

Difflugia, feeding of, 136.

DONNELLON, JAMES A. An experimental study of clot formation in the perivisceral fluid of Arbacia (abstract), 337.

DOYLE, W. L., AND C. W. METZ. Structure of the chromosomes in the salivary gland cells in Sciara (Diptera), 126.

DREYER, W. A. Water content of insects in relation to temperature and humidity (abstract), 338.

DUSHKIN, M. A. *See* Bachem and Dushkin, 109.

ELODEA leaves, indirect effects of radiation, 279.

Eumeces laticeps, effects of antuitrin-S on male genital organs during seasonal atrophy, 143.

FEEDING habits and pharyngeal structure in Stenostomum, 439.

—— of Difflugia, Pontigulasia, and Lesquereusia, 136.

FERGUSON, J. K. W. The scope of the chemical method of estimating hemoglobo-carbamate (abstract), 327.

Fertilizable period of eggs of Fundulus, 185.

Fertilization membrane, origin and nature, in various marine ova, 159.

—— reaction, temperature effect, in marine ova, 415.

FRIEDMANN, HERBERT. Notes on differential threshold of reaction to vitamin D deficiency in the house sparrow and the chick, 71.

Fundulus, fertilizable period of eggs, 185.

——, melanophore-dispersing principle in hypophysis, 379.

GRAFFLIN, ALLAN L. Chloride and total osmotic pressure in the blood of marine teleosts, 245.

——, —— —. Renal function in marine teleosts, 391.

GUTTMAN, RITA. Differential oxygen uptake of regions of Limulus optic nerve as related to distance from the sense organ (abstract), 342.

——, ——. Differential oxygen uptake of regions of Limulus optic nerve as related to distance from the sense organ, 356.

HAIG, CHARLES. The phototropic responses of Avena in relation to intensity and wave-length, 305.

HARVEY, ETHEL BROWNE. Parthenogenetic merogony or cleavage without nuclei in Arbacia punctulata (abstract), 332.

——, —— ——. *See* Navez and Harvey (abstract), 342.

——, —— ——. Some surface phenomena in the fertilized sea urchin egg influenced by centrifugal force, 298.

——, —— ——. The mitotic figure and cleavage plane in the egg of Parechinus microtuberculatus, as influenced by centrifugal force, 287.

HARVEY, E. N. *See* Hunter and Harvey (abstract), 344.

HAYWOOD, CHARLOTTE, AND RUDOLF HÖBER. The permeability of the frog liver to certain lipoid insoluble substances (abstract), 340.

HEILBRUNN, L. V. Protein lipid binding in protoplasm (abstract), 329.

——, —. —., AND KATHRYN DAUGHERTY. Indirect effects of radiation on Elodea leaves, 279.

——, —. —., AND R. A. YOUNG. Indirect effects of radiation on sea urchin eggs, 274.

Hemolymph cell counts of some marine invertebrates, 66.

HENSHAW, PAUL S. A consideration of biological factors influencing the radio-sensitivity of cells (abstract), 328.

HESS, WALTER N. Light reactions and photoreceptors of Dolichoglossus Kawalevskyi (abstract), 336.

HIBBARD, HOPE, AND ROBERT CHAMBERS. Micromanipulation of egg and nurse cells in Bombyx mori (abstract), 332.

HÖBER, RUDOLF. *See* Haywood and Höber (abstract), 340.

HODGE, CHARLES. The anatomy and histology of the alimentary tract of the grasshopper, Melanopus differentialis Thomas (abstract), 338.

HOLTER, HEINZ. Localization of peptidase activity in the eggs of Echinarachnius and Arbacia (abstract), 333.

HUGHES-SCHRADER, SALLY. The chromosome cycle of Phenacoccus (Coccidae), 462.

HUNTER, F. R., AND E. N. HARVEY. The effect of lack of oxygen on the permeability of the egg of Arbacia punctulata to ethylene glycol (abstract), 344.

Hypophysis, melanophore-dispersing principle in, of Fundulus heteroclitus, 379.

INTERSEXES, annulus ventralis, in Cambarus, 269.

Intranuclear achromatic figure of Oxymonas grandis sp. nov., 54.

Iodacetate, influence on activation and development of eggs of Arbacia punctulata, 351.

JACOBS, M. H., AND A. K. PARPART. The permeability of the erythrocyte to ammonia (abstract), 344.

——, —. —., AND DOROTHY R. STEWART. The manner of entrance of ammonium salts into cells (abstract), 330.

JOHNSON, MARTIN W. Seasonal migrations of the wood-borer Limnoria lignorum (Rathke) at Friday Harbor, Washington, 427.

JUDAY, C., AND H. A. SCHOMER. The utilization of solar radiation by algae at different depths in lakes, 75.

KAGAN, BENJAMIN M. The fertilizable period of the eggs of Fundulus heteroclitus and some associated phenomena, 185.

KELTCH, A. K. *See* Clowes, Krahl, and Keltch (abstract), 341.

KESSLER, WALTER A. *See* Dawson, Kessler, and Silberstein, 447.

KILLE, FRANK R. Regeneration in Thyone briareus Lesueur following induced autotomy, 82.

KLEINHOLZ, L. H. The melanophore-dispersing principle in the hypophysis of Fundulus heteroclitus, 379.

KRAHL, M. E. *See* Clowes, Krahl, and Keltch (abstract), 341.

——, —. —., AND G. H. A. CLOWES. Effect of nitrophenols and related compounds on metabolism of living cells (abstract), 340.

LESQUEREUSIA, feeding of, 136.

Ligia, salt requirements and orientation, 259.

Limnoria lignorum, seasonal migrations at Friday Harbor, Washington, 427.

Limulus, optic nerve, differential oxygen uptake, 356.

Lizard, effects of antuitrin-S on male genital organs during seasonal atrophy, 143.

MANTIS, praying, sex behavior, 203.

Marine Biological Laboratory, program and abstracts of papers presented in 1935, 325.

—— —— ——, thirty-seventh report, 1.

MCBRIDE, THOMAS F. *See* Wolf and McBride (abstract), 334.

——, —— —., AND E. ALFRED WOLF. Early stages of calcification in teeth (abstract), 334.

Melanophore-dispersing principle in hypophysis of Fundulus heteroclitus, 379.

METZ, C. W. *See* Doyle and Metz, 126.

Migrations, seasonal, of wood-borer Limnoria lignorum at Friday Harbor, Washington, 427.
Mitosis, Amoeba dubia, 447.
——, centrioles of Pseudotrichonympha and their rôle in, 46.
Mitotic figure and cleavage plane, influence of centrifugal force on, 287.
Moser, Floyd. Changes at the surface of Arbacia punctulata eggs during membrane elevation (abstract), 333.

Navez, Albert E., and Ethel Browne Harvey. Indophenol oxidase activity in intact and fragmented Arbacia eggs (abstract), 342.
Nitrogen, determination in plankton, 221.
Nuttycome, John W., and Aubrey J. Waters. Feeding habits and pharyngeal structure in Stenostomum, 439.

Optic nerve, Limulus, differential oxygen uptake, 356.
Orientation of Ligia in Bermuda, 259.
Osmotic pressure and chloride in blood of marine teleosts, 245.
Osterhout, W. J. V. The rôle of ions in Valonia and in Nitella (abstract), 329.
Oxygen uptake, differential, of regions of Limulus optic nerve as related to distance from sense organ, 356.
Oxymonas grandis sp. nov., intranuclear achromatic figure, 54.

Palmer, Louise. The shedding reaction of Arbacia punctulata (abstract), 336.
Parpart, A. K. *See* Jacobs and Parpart (abstract), 344.
Patrick, Ruth. Some diatoms of the Great Salt Lake as indicators of present and geological water conditions (abstract), 338.
Pharyngeal structure and feeding habits in Stenostomum, 439.
Phenacoccus, chromosome cycle, 462.
Phototropic responses, Avena, in relation to intensity and wave-length, 305.
Plankton, nitrogen and carbon determination in small amounts, 221.
Polar bodies, giant, formation of, in centrifuged eggs of Ilyanassa, 403.
Pontigulasia, feeding of, 136.
Program and abstracts of papers presented at scientific meetings, Marine Biological Laboratory, 325.
Prosser, C. Ladd. *See* Sichel and Prosser (abstract), 343.
Pseudotrichonympha, centrioles and rôle in mitosis, 46.

Radiation, indirect effects on Elodea leaves, 279.
——, indirect effects on sea urchin eggs, 274.
——, solar, utilization by algae at different depths in lakes, 75.
Renal function, marine teleosts, 391.
Regeneration in Thyone briareus Lesueur following induced autotomy, 82.
Respiratory metabolism in fishes and susceptibility to anesthetics, 368.
—— —— in teleost fish, as affected by acclimatization to high and low temperatures, 361.
Richards, Oscar W. Killing organisms with chromium as from incompletely washed bichromate-sulphuric cleaned glassware (abstract), 327.
Roehder, K. D. An experimental analysis of the sexual behavior of the praying mantis (Mantis religiosa L.), 203.
Runnstrom, John. Acid formation in frozen and thawed Arbacia punctulata eggs and its possible bearing on the problem of activation, 345.
——, ——. Influence of iodoacetate on activation and development of the eggs of Arbacia punctulata, 351.

Salinity, effects on body weight and survival of Nereis, 233.
Salivary gland cells, chromosome structure, in Sciara, 126.
Salt requirements and orientation of Ligia, in Bermuda, 259.
Sayles, Leonard P. The effects of salinity changes on body weight and survival of Nereis virens, 233.
Schomer, H. A. *See* Juday and Schomer, 75.
Sciara, chromosome structure in salivary gland cells, 126.
Sea urchin egg, influence of centrifugal force on surface phenomena, 298.
— —— —, indirect effects of radiation on, 274.
Sex, behavior of praying mantis, 203.

—, effects of antuitrin-S on male genital organs of lizard during seasonal atrophy, 143.

—, secondary characters, crayfishes, 269.

SICHEL, F. J. M., AND C. LADD PROSSER. Spatial relations in the excitation of the isolated muscle fibre (abstract), 343.

SILBERSTEIN, JOSEPH A. *See* Dawson, Kessler, and Silberstein, 447.

Solar radiation, utilization, by algae at different depths in lakes, 75.

SPEIDEL, CARL CASKEY. Effects of alcohol on nerves (abstract), 325.

——, — ——. Successive injury and recovery of striated muscle (abstract), 325.

STANBURY, JOHN, AND WILLIAM R. AMBERSON. The rate of regeneration of the blood proteins after complete exsanguination (abstract), 339.

Stenostomum, feeding habits and pharyngeal structure, 439.

STEWART, DOROTHY R. *See* Jacobs and Stewart (abstract), 330.

STIX, HELEN. *See* Tashiro and Stix (abstract), 327.

STUMP, A. B. Observations on the feeding of Difflugia, Pontigulasia, and Lesquereusia, 136.

SUMNER, F. B., AND N. A. WELLS. Some relations between respiratory metabolism in fishes and susceptibility to certain anesthetics and lethal agents, 368.

Survival, as influenced by changes in salinity, Nereis, 233.

Susceptibility to anesthetics, and respiratory metabolism in fishes, 368.

TASHIRO, SHIRO, AND HELEN STIX. Dacryorrhetin: Demonstration of its action by a kodachrome film, (abstract), 327.

TAUBER, OSCAR E. *See* Yeager and Tauber, 66.

Teleosts, marine, renal function in, 391.

Temperature, effect on rate of fertilization reaction in various marine ova, 415.

TEORELL, TORSTEN. Some aspects of elektrolyte diffusion (abstract), 331.

Thyone briareus Lesueur, regeneration in, following induced autotomy, 82.

TURNER, C. L. Studies on the secondary sexual characters of crayfishes. X. The annulus ventralis in true intersexes of Cambarus, 269.

TURNER, CLARENCE D. The effects of antuitrin-S on the male genital organs of the lizard (Eumeces laticeps) during seasonal atrophy, 143.

ULTRAVIOLET radiation, bacterial sensitivity to, 109.

VITAMIN D deficiency, differential threshold of reaction to, in house sparrow and chick, 71.

WATERS, AUBREY J. *See* Nuttycombe and Waters, 439.

Weight, as affected by changes in salinity, Nereis, 233.

WELLS, NELSON A. Change in rate of respiratory metabolism in a teleost fish induced by acclimatization to high and low temperature, 361.

——, —— —. *See* Sumner and Wells, 368.

WOLF, E. ALFRED. *See* Wolf and McBride (abstract), 334.

——, —. ——, AND THOMAS F. MCBRIDE Early stages of ossification (abstract), 334.

YEAGER, J. FRANKLIN, AND OSCAR E. TAUBER. On the hemolymph cell counts of some marine invertebrates, 66.

YOUNG, R. A. *See* Heilbrunn and Young, 274.

MARINE BIOLOGICAL LABORATORY

SUPPLY DEPARTMENT

WOODS HOLE, MASS.

◆

For a number of years the Supply Department has been furnishing Living Marine Materials. From experience it has been found that during the period between the first of November and the end of February these live animals and plants can be shipped with the most success. This service has proven to be of great value to both instructor and student, and we have had numerous letters of commendation on our Live Marine Material. We would be very glad to have the opportunity of sending one of these sets to your school or college. Instructions regarding these shipments will be gladly sent upon request, and shipments are made from November through February with the guarantee that they will ship in good condition as far west as the Mississippi and as far south as Georgia.

Regarding the Dogfish material, we believe we can state without contradiction that those specimens prepared by the Marine Biological Supply Department are superior to any on the market. The long experience of the preparators and the excellent boat and laboratory equipment enable us to handle large numbers of Dogfish during the period when they are migrating, and we have a complete stock of all sizes of plain and injected spiny Dogfish, heads for brain study and specimens in brine for skeletons.

All materials purchased from us are guaranteed to give satisfaction, and any items which are found to be otherwise may be returned for full credit at our expense.

Catalogues on request

◆

The Marine Biological Laboratory takes this opportunity of extending to its many patrons

The Season's Greetings

CONTENTS

Page

RUNNSTRÖM, JOHN
Acid Formation in Frozen and Thawed Arbacia punctulata Eggs and its Possible Bearing on the Problem of Activation . . 345

RUNNSTRÖM, JOHN
Influence of Iodoacetate on Activation and Development of the Eggs of Arbacia punctulata 351

GUTTMAN, RITA
Differential Oxygen Uptake of Regions of Limulus Optic Nerve as Related to Distance from the Sense Organ 356

WELLS, NELSON A.
Change in Rate of Respiratory Metabolism in a Teleost Fish Induced by Acclimatization to High and Low Temperature . . 361

SUMNER, F. B., AND N. A. WELLS
Some Relations between Respiratory Metabolism in Fishes and Susceptibility to Certain Anesthetics and Lethal Agents . . 368

KLEINHOLZ, L. H.
The Melanophore-dispersing Principle in the Hypophysis of Fundulus heteroclitus 379

GRAFFLIN, ALLAN L.
Renal Function in Marine Teleosts 391

CLEMENT, ANTHONY C.
The Formation of Giant Polar Bodies in Centrifuged Eggs of Ilyanassa 403

CHASE, H. Y.
The Effect of Temperature on the Rate of the Fertilization Reaction in Various Marine Ova 415

JOHNSON, MARTIN W.
Seasonal Migrations of the Wood-borer Limnoria lignorum (Rathke) at Friday Harbor, Washington 427

NUTTYCOMBE, JOHN W., AND AUBREY J. WATERS
Feeding Habits and Pharyngeal Structure in Stenostomum . . 439

DAWSON, J. A., WALTER R. KESSLER, AND JOSEPH K. SILBERSTEIN
Mitosis in Amoeba dubia 447

HUGHES-SCHRADER, SALLY
The Chromosome Cycle of Phenacoccus (Coccidae) 462

SMITHSONIAN INSTITUTION LIBRARIES
3 9088 01228 1523